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A Comparison of Genetic Identifications and Pigment Patterns of *Sebastes* Larvae Caught on NOAA Ship *John N. Cobb* Cruise 9809

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Abstract

During a cruise in Southeast Alaska in July 1998, *Sebastes* larvae were collected by plankton net, immediately sorted from the net, their pigment characters noted, photographed with a digital camera, and preserved for later genetic analysis. From these collections, 103 preflexion larvae had their pigment characters noted, 84 were subjected to genetic analysis (polymerase chain reaction followed by restriction fragment length analysis of the **ND3/ND4** region of mtDNA), and 67 were subjected to both pigment and genetic analysis. These larvae were grouped into eight pigment groups. The genetic analysis found seven species. With few exceptions, there was poor agreement between the pigment groups and the genetic identity of these larvae. The four larvae of *Sebastes maliger/caurinus* all shared dorsal postanal melanophores (pigment group 1), that were not present on any other genetically identified larvae. Pigment group 8 contained only six specimens of *Sebastes zacentrus*; however, the five other *S. zacentrus* that were identified were in four other pigment groups. Based on this study, identification to species of preflexion larvae of *Sebastes* from the northeast Pacific on the basis of mtDNA analysis is effective; however, identification on the basis of pigment patterns remains problematic.

Introduction

Preflexion larvae of *Sebastes*, rockfishes, are abundant in plankton collections in temperate and subarctic waters of the Pacific Ocean and adjacent marine waters in spring and summer. These larvae are all about the same size (3-5 mm) and body shape and are easily identified as members of the genus *Sebastes* (Moser et al. 1977, Matarese et al. 1989, Moser 1996). However, as many as 20 species of *Sebastes* may occur in one locality in this region (Orr et al. 2000), and identification of these larvae to species, except for a few species, is not yet possible. All are rather lightly pigmented (with melanophores) and share pigment in some areas, while pigment is variably present in other areas. These pigment patterns seem to be the only possible characters that are microscopically visible on these larvae that might have diagnostic value. Since *Sebastes* are viviparous, obtaining live hatchling larvae is not difficult, and preflexion larvae of most species have been illustrated and described. Such studies have shown that there is considerable within species variability in pigment patterns (Marliave et al. 1997), and larvae of several species can share identical pigment patterns (Kendall 1991). Rearing *Sebastes* larvae beyond the preflexion stage has been successful for only a few species, so identification of larger larvae in plankton samples is generally not possible either.

Application of genetic techniques that require minute quantities of tissue seem to offer a means to overcome these problems with identifying *Sebastes* larvae (Seeb and Kendall 1991, Rocha-Olivares 1998). Once genetic criteria are established to identify the adults of *Sebastes* species, these criteria can be applied to larvae (Taylor 1997). Genetic criteria are available for almost all species of *Sebastes* (A. J. Gharrett, School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks, 11120 Glacier Highway, Juneau, AK 99801, pers. comm.), and tests have confirmed that these criteria can be applied to larvae. However, there are at least two problems in applying these techniques to routine identification of larvae. The larvae are macerated in preparation for genetic analysis and so are not available for further study (e.g., otolith ageing, food habits, morphological examination). These techniques also require that the larvae be sorted

from the plankton samples immediately and individually placed in preservative for analysis. In many studies, it is not possible to handle the larvae in this way aboard a ship.

Thus, it is still worthwhile to attempt to find ways to identify field-caught *Sebastes* larvae microscopically without destroying them for other studies. The present study combined an analysis of pigment patterns and genetic identification of preflexion *Sebastes* larvae collected in July in Southeast Alaska, in an area where several species would be expected to be releasing larvae. The objective was to examine within and between species variability in pigment patterns of preflexion field-caught larvae whose identity had been established genetically.

Methods and Materials

A series of 26 Tucker trawl tows was made near Chatham Strait in Fredrick Sound in Southeast Alaska (Fig. 1) on 6-7 July 1998 aboard the NOAA ship *John N. Cobb*. The net was towed during daylight at about 0.8 m/sec at various depths (usually about 5 m) for 5 min. Once the net was retrieved, the codend was emptied into a chilled white sorting tray and all visible *Sebastes* larvae were removed as quickly as possible. Larvae were then individually labeled and photographed with a digital camera. Pigment of each larva was then quickly sketched on a template drawing of a preflexion *Sebastes* larva. Larvae were then put into 2 ml tubes containing a DNA preservation solution (20% dimethyl sulfoxide [DMSO], 0.25 M ethylenediamine-tetraacetic acid [EDTA], pH 8, NaCl saturated) (Seutin et al. 1991) for later genetic analysis.

Later, in the laboratory on shore, the sketches of pigment were examined to see what possible morphological areas were pigmented on any of the larvae. This resulted in a modification of the pigment loci used by Kendall (1991). A total of 15 areas was pigmented on some of the larvae collected in this study (Fig. 2). Each larva was then scored on whether it was pigmented in each of these areas. These data were then analyzed and eight pigment groups were established (Table 1). Larvae were then assigned to these pigment groups. Some larvae from each pigment group

were selected for genetic analysis, which was performed without knowledge of the pigment group assignments. The genetic identifications of these larvae were then compared with the pigment groups to which the larvae had been assigned.

For genetic analysis, total cellular DNA was isolated from individual larvae using a Puregene™ DNA isolation kit (Gentra Systems, Inc.). The NADH-dehydrogenase-3 and NADH-dehydrogenase-4 (**ND3/ND4**) region of the rockfish mitochondrial DNA (mtDNA) was amplified by polymerase chain reaction (PCR) from total cellular DNA using primers that were developed for coho salmon (*Oncorhynchus kisutch*) and adapted for rockfish mtDNA studies (Gharrett et al. in press.). The conditions for amplification were: an initial denaturation at 94°C for 5 min, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, and 3 min at 72°C using Taq polymerase from Perkin Elmer [1](Norwalk, CT) according to manufacturer's directions.

Amplified regions were digested with restriction endonucleases *Bst*N I, *Dde* I, *Mbo* I, *Msp* I and *Rsa* I (Gharrett et al. in press) according to manufacturer's instructions. The digested fragments were separated by electrophoresis through a 1.5% mixture composed of one part Ultra Pure™ agarose (BRL Gibco, Grand, NY) and two parts Synergel™ (Diversified Biotech Inc., Boston, MA) in 0.5 X TBE buffer (TBE is 90 mM Tris-HCl, 90 mM boric acid, and 2 mM EDTA, pH 8.3). DNA bands were stained with ethidium bromide and photographed on an ultraviolet light transilluminator. A 100 bp ladder was used as molecular weight markers to estimate restriction fragment sizes.

Results

Pigment patterns were sketched for 103 preflexion larvae (Appendix 1). Pigment was found to occur in 15 different areas on these larvae (Fig. 2). These areas corresponded to most of those used by Kendall (1991); however, two additional areas were defined in this study, and locus 26

[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

was subdivided into two loci: an anterior one and a posterior one. All larvae had the dorsal surface of the gut pigmented (locus 10), and all had a row of ventral postanal melanophores (locus 12). None had pigment on their pectoral fins. Few larvae shared identical pigment patterns, but eight general patterns were established (Table 2). These pigment groups contained from 4 to 37 larvae. All groups except 1 and 8 had some hypural pigment (loci 13, 14, 15). Group 1 contained 5 larvae and these were the only larvae that had dorsal trunk pigment (locus 4), prethoracic cavity pigment (locus 6), and the ventral postanal midline series originating at the vent (locus 11). Other studies have shown that this pattern is characteristic of the subgenus *Pteropodus* (Kendall 1991). Group 2 had 18 specimens all with a dorsal hypural spot (locus 5). Group 3 contained 10 larvae that had lower jaw pigment (locus 1), but no dorsal hypural spots (locus 5) where group 2 was pigmented, or dorsal postanal spots (locus 4) where group 1 was pigmented. Group 4 with 14 specimens, had cranial (locus 2) and nape (locus 3) pigment, but no lower jaw (locus 1) or other dorsal pigment. Group 5 had 7 larvae with cranial (locus 2), but no nape (locus 3) pigment. Group 6 consisted of 4 larvae with nape (locus 3), but no cranial (locus 2) pigment. Group 7 with 37 specimens, had no head (loci 1, 2) or nape (locus 3) pigment, but did have some hypural (loci 13, 14, or 15) pigment. Group 8 with 8 larvae was the least pigmented group. In addition to the pigment shared by all larvae (loci 10, and12), they had some ventral gut pigment (loci 8, 9). They had no head (loci 1, 2, 3) or hypural pigment (loci 13, 14, 15).

We conducted restriction analyses on the **ND3/ND4** region of mtDNA of 84 larval rockfish. The enzymes chosen for analysis (*Bst*N I, *Dde* I, *Mbo* I, and *Rsa* I) produce restriction fragment patterns with which we have been able to distinguish among 15 species of adult rockfish that are common in waters off Southeast Alaska (Table 3).

We performed preliminary species identification by examining the composite restriction fragment patterns of each larva and comparing them with adult reference data (Table 4). Digests of each larva were subsequently run next to the digest of a known adult rockfish to confirm species identification. Larvae which did not have composite restriction fragment patterns

identical to the adult reference data were cut with *Msp* I to increase the amount of information in the composite haplotype. Subsequently we digested samples that did not resemble references with *Dde* I.

Seventy-three of the larvae had restriction fragment patterns identical to those of adult specimens previously observed. We identified 3 *S. maliger/caurinus*, 5 *S. brevispinis*, 7 *S. proriger*, 12 *S. ruberrimus*, 31 *S. variegatus*, and 14 *S. zacentrus* (Table 3). The other nine larvae had restriction fragment patterns that differed by a single site from patterns that we observed in our limited adult rockfish reference survey. Larvae T2F5, T18F5, and T19F5 were one *Bst*N I site different from *S. variegatus*. Larvae T16F5, T19F5, T23F13 and T2F7 differed at one *Dde* I site with *S. variegatus*. Larvae T7F3 and T24F2 had one *Dde* I site difference from *S. zacentrus* and *S. ruberrimus*, respectively. Larvae T8F1 and T4F1 differed at one *Dde* I site with *S. babcocki*. Larva T25F3 was one *Rsa* I site different from *S. maliger*. For these larvae all other restriction endonucleases fragment patterns were identical to adult reference patterns. Larva T1F1 has a composite restriction fragment pattern not previously observed in any adult rockfish that we have examined.

Presumably, larvae possessing a single loss or gain of a restriction site exhibit intraspecific variation not previously observed. This assumption is based on a phylogenetic study of *Sebastes* in which we mapped restriction sites in the **ND3/ND4** and **12S/16S** regions of the mtDNA. Phenetic restriction maximum likelihood (RML) and cladistic parsimony trees demonstrated that intraspecific variation resulted in terminal branching and did not interfere with interspecific branching (Gharrett and Gray unpublished data).

A total of 67 of the larvae whose pigment patterns had been sketched were identified genetically (Table 2). Among these, 7 species were found, and one larvae could not be identified. *S. variegatus* was represented by the most larvae, 29. There were 11 larvae of both *S. ruberrimus* and *S. zacentrus*. *S. proriger* had 5 larvae, and *S. brevispinis* and *S. maliger/caurinus* each had 4. *S. babcocki* had 2 larvae.

When comparing the genetic identifications with the pigment groups, it is evident that in most cases there is considerable intraspecific variability in pigment (Table 6). All of the *S. maliger/caurinus* were in pigment group 1, which was quite distinctive being the only group with a dorsal postanal series of melanophores. This is characteristic of all of the species of the subgenus *Pteropodus* for which the preflexion larvae are known. It was not possible to differentiate between *S. maliger* and *S. caurinus* on the basis of larval pigment or the genetic tests that we performed. Had other species of *Pteropodus* been present, *S. maliger/caurinus* may have been confused with them. The other species that seemed closely related to a pigment group was *S. zacentrus*; all 6 members of pigment group 8 were identified as *S. zacentrus*. However, among the 11 larvae that were identified as *S. zacentrus*; the five that were not in group 6 were in four other pigment groups. In looking at the sketches of pigment for those larvae identified as *S. zacentrus*, it appears that the ventral postanal melanophore series is shorter than it is in other species. *S. zacentrus* larvae that were not in group 6 had additional pigment in several areas: ventral gut, cranial, nape, lower jaw, and hypural. Previously published illustrations of *S. zacentrus* extrusion larvae do not show any head pigment (Westrheim et al. 1968b, Efremenko and Lisovenko 1970, Harling et al. 1971, Matarese et al. 1989). *S. babcocki* larvae were in pigment groups 3 and 4: they both had nape and hypural pigment, but there were differences in head pigment between the two larvae. A previously published illustration of a preflexion larva of *S. babcocki* shows a dorsal postanal series of melanophores and considerable ventral gut pigment, but no head pigment (Westrheim et al. 1968a). Three *S. brevispinus* were in group 7 and one was in group 3. All had ventral gut pigment and hypural pigment on the body, but only one had lower jar pigment. Previously published illustrations of extrusion larvae of *S. brevispinus* (DeLacy et al. 1964, Matarese et al. 1989) show pigment patterns similar to those seen on the larvae in this study. *S. proriger* larvae were in 3 pigment groups: one in group 2, three in group 6 and one in group 7. They all had ventral gut pigment just anterior to the vent, and hypural pigment on the body. Three had nape pigment and one had cranial pigment. A previously published illustration of an extrusion larva of *S. proriger* shows a similar pigment pattern, except no head pigment is evident (Westrheim et al. 1968b). *S. ruberrimus* had two larvae in group 3, seven in group 4 and two in group 7. They all had hypural pigment on the

body and considerable ventral gut pigment. All but one had pigment on the cranium or nape or both. Previously published illustrations of extrusion larvae of *S. ruberrimus* show considerable variation in pigment: some have hypural pigment and some do not, and there is variation in the amount and placement of gut pigment (DeLacy et al. 1964, Westrheim et al. 1968b, Harling et al. 1971, Kendall 1989). The 29 specimens of *S. variegatus* were in 5 pigment groups. Group 7 contained the most *S. variegatus* (13). All had hypural pigment and pigment just anterior to the vent. Head pigment was variable, but most had none. Previously published illustrations of extrusion larvae of *S. variegatus* show similar pigment patterns to those seen in the larva in this study (Harling et al. 1971, Matarese et al. 1989).

Discussion

The sizes of most populations of *Sebastes* in the northeast Pacific are greatly reduced because of overfishing and environmental impacts(Mason 1998, Love 1998a, 1998b, Woodbury 1999). Understanding population processes in these many species, and assessing their abundance are major fisheries issues in this area (Parker et al. 2000). Successful management and rebuilding of these stocks requires such knowledge. The larval stage of *Sebastes* offers an opportunity to provide an independent measure of population size and can lead to an understanding of recruitment mechanisms and year-class variation. However, our inability to identify most larval *Sebastes* to the species level precludes their use in such studies.

Sebastes demonstrate an unusual reproductive mode among fishes in that they have internal fertilization and brood large numbers (~10^5) of larvae through most of the yolk sac stage. After brooding for several weeks and obtaining some nourishment from the female, larvae are released at a feeding-competent stage (Kendall and Lenarz 1987). *Sebastes* larvae are usually among the most abundant taxa found in ichthyoplankton collections in the northeast Pacific and adjacent marine waters in spring and summer (Doyle 1992, Moser 1996). Most *Sebastes* larvae in these collections are readily recognized as members of the genus but are small (preflexion and early flexion) and have not yet developed distinguishing adult characteristics. Only a few species

have been found to have distinguishing larval characters (see Matarese et al. 1989, Moser 1996). Most are nearly identical morphologically and can be identified only to genus, which precludes their use in detailed studies, since in most areas, based on the distribution of adults and the timing of release of larvae, the larvae of several species are expected to co-occur in the same sample.

Several studies have attempted to define the pigment characteristics of preflexion *Sebastes* larvae in order to use pigment to identify field-caught larvae (e.g., Waldron 1968, Westrheim 1975). Most of these studies have used full-term larvae extruded from adults and have tried to rear the larvae for some time. With relatively few exceptions (e.g., Moser and Butler 1987, Moreno 1993), rearing has been unsuccessful in that the larvae fail to eat and die within a few days when they exhaust their yolk supplies. A consistent result of these studies is that there is considerable variability in pigment within broods of larvae and also considerable overlap in pigment among species. This prevents specific identification of larvae in field samples based on pigment.

A further complication is that larval pigment changes and moves during the yolk sac and preflexion stages. Areas that were unpigmented when the larvae were first released can become pigmented over the next few days. Other morphological changes during this period are hardly discernable, but larvae of the same species can have different pigment patterns just because of a few day's difference in the age of the larvae. Most larvae in the present study were in the preflexion stage and were probably only a few days past extrusion. However, differences between the pigment patterns observed here and those in previously published illustrations of extrusion larvae could be partially due to differences in ages of the larvae.

In the present study, the identity of field-caught preflexion *Sebastes* larvae was established genetically. The pigment characters of these larvae were described independently, and then groupings of larvae, based on pigment characters were compared with the genetic identifications. As in previous studies, based on extruded larvae from known adults, we found that larvae of the

same species were generally variable in pigment. Of the seven species identified by genetics, two had larvae with some distinguishing larval pigment patterns. *S. maliger/caurinus* was the only species group whose larvae had a series of dorsal postanal midline melanophores. Larval descriptions of *S. maliger* in the literature are incomplete, and it is not possible to separate them from other species of *Sebastes*, particularly in the subgenus *Pteropodus* (e.g., *S. caurinus* and *S. auriculatus*: see Stahl-Johnson 1985). DeLacy et al. (1964), Westrheim et al. (1968b) and Kendall (1996) described preflexion *S. maliger* larvae extruded from known adults. Attempts to rear these larvae beyond the preflexion stage were unsuccessful, and later larvae have not been described. *S. maliger/caurinus* was the only member of the subgenus *Pteropodus* identified in the present samples. Based on other studies, other species of *Pteropodus* also have dorsal postanal midline melanophores. Thus, this might be a characteristic of this subgenus and not diagnostic for *S. maliger/caurinus* alone.

The other species that had somewhat distinctive larvae was *S. zacentrus*. The ten larvae identified genetically as *S. zacentrus* had pigment at fewer loci than larvae of most other species. Also, the ventral postanal melanophore series seemed to be shorter in *S. zacentrus* than it was in other species. This characteristic was seen in examining the pigment sketches but was not noted at the time the sketches were made. Thus, it could not be quantified or defined precisely. All but one specimen of *S. zacentrus* had some ventral gut pigment. Only three specimens of *S. zacentrus* had any head pigment. Larvae of *S. zacentrus* examined here agreed closely in pigment with those illustrated in the literature (see Westrheim et al. 1968b, Efremenko and Lisovenko 1970, Harling et al. 1971, Matarese et al. 1989).

Although all larvae examined here had a ventral postanal series of melanophores and dorsal pigment on the gut, pigment at most other loci was variably present among the larvae of a species. No particular pigment locus seemed diagnostic for a species (except see discussion of *S. maliger*). All species showed some variability in the presence of pigment at most loci. It is unknown how much of this variability is due to the age of the larvae, and how much of it is just inherent within-species variability in the distribution of melanophores.

Interspecific variation in mtDNA provides a tool that can be used to advance our knowledge of rockfish biology in several areas. First, it will make it possible to conduct surveys of larval abundance in which most rockfish larvae can be identified to species. Such studies would make important contributions to marine ecological studies and fisheries stock assessments. Second, it will make it possible to fill many gaps in our knowledge of the early life history of rockfish species that exist because of the difficulty in identifying larval and juvenile rockfish. It will also make it possible to detail morphological changes that accompany development and metamorphosis of rockfish and which may ultimately provide morphological cues that can be used to identify small rockfishes.

In summary, at least for the species of *Sebastes* identified here on the basis of genetics, pigment patterns on the preflexion larvae do not appear to offer an effective way of identifying the species of larvae in field collections.

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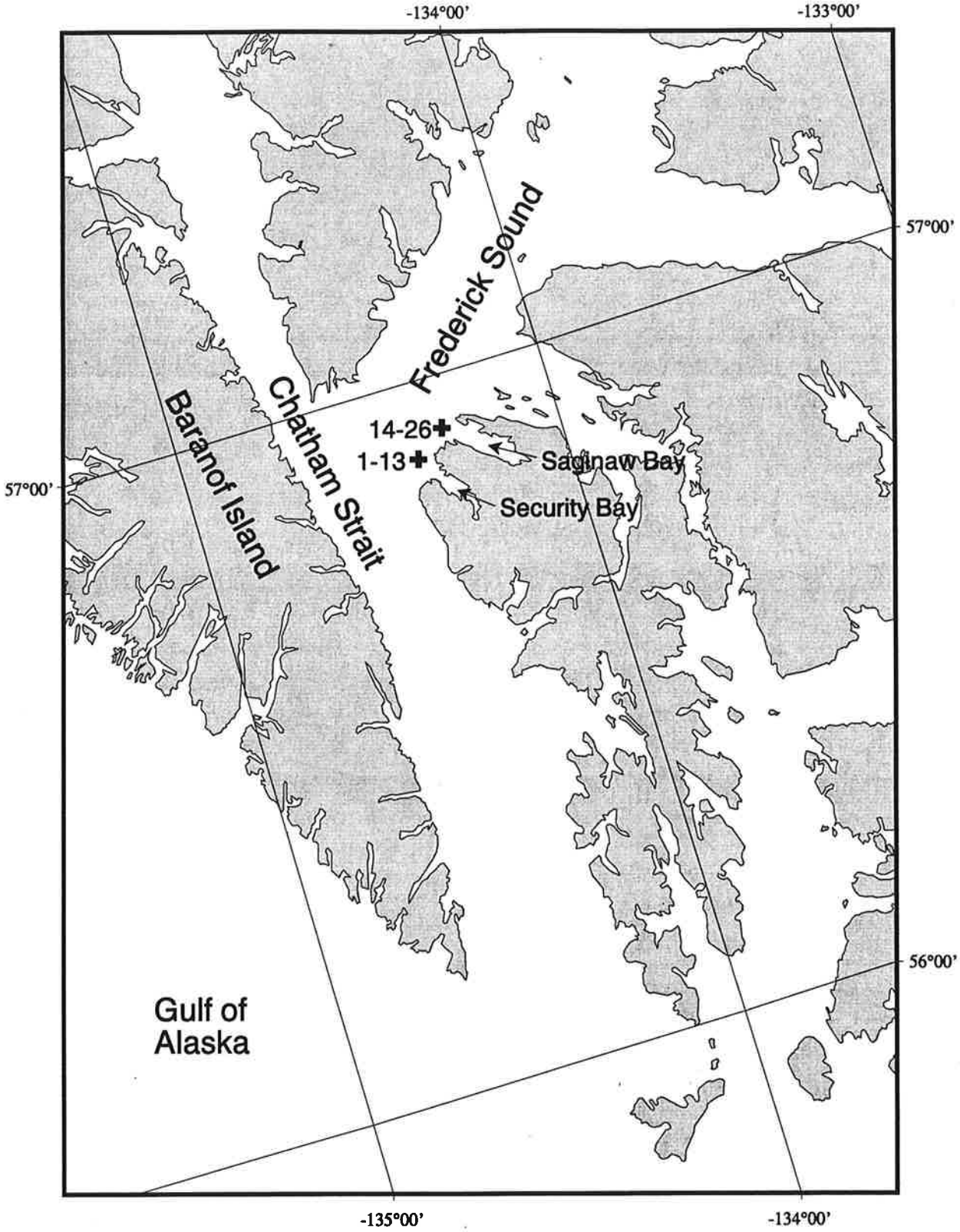

Figure 1. Collection locations for larvae used in this study. Tows 1-13 were made off Security Bay, tows 14-26 were made off Saginaw Bay.

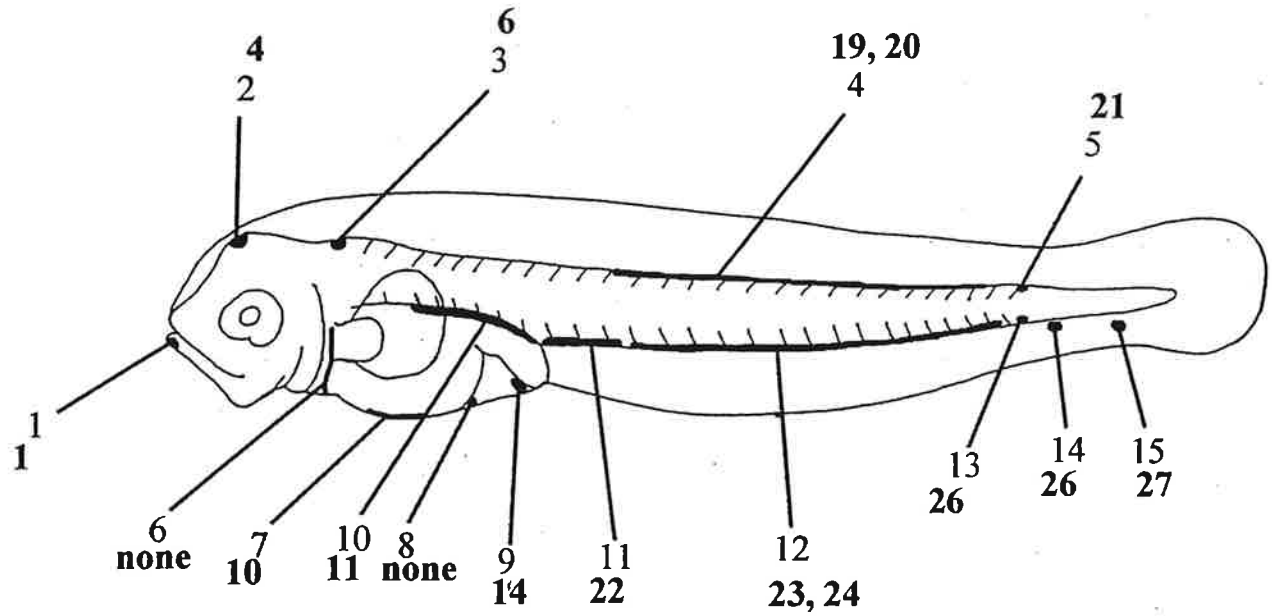

Figure 2. Preflexion *Sebastes* larval pigment loci used in this study in comparison with those used by Kendall (1991). Pigment loci used in this study are indicated by regular font numbers, those used by Kendall (1991) are indicated by bold font numbers.

Table 1. Major characteristics of pigment groups.

Pigment Group	Number of Specimens	Pigment Characteristics
1	5	*Pteropodus* type: dorsal trunk pigment, postanal ventral midline pigment starts at vent.
2	18	Dorsal hypural spot present.
3	10	Lower jaw spot present, no dorsal midline spots.
4	14	Cranial and nape pigment present, no lower jaw or other dorsal pigment, some hypural spots.
5	7	Cranial but no nape pigment.
6	4	Nape but no cranial pigment.
7	37	No head or nape pigment, hypural pigment present.
8	8	No head or nape pigment, no hypural pigment.

Table 2. Pigment characters of genetically identified larvae.

Species	Number of Specimens	Pigment Characteristics
S. babcocki	2	Both have dorsal gut, postanal ventral, nape, hypural (finfold and body), one has crown, one has lower jar tip, one has ventral gut.
S. brevispinis	4	All have dorsal gut, postanal ventral, 2 or 4 hypural on body, ventral gut, one has lower jar tip.
S. maliger	4	All have dorsal gut, postanal ventral (extending forward to gut), nape and posterior crown, ventral gut just anterior to anus and forward along gut and anterioventral on gut, postanal dorsal, two have lower jar tip.
S. proriger	5	All have dorsal gut, postanal ventral, 1 or 2 hypural on body, ventral gut just anterior to anus, three have nape, one has crown.
S. ruberrimus	11	All have dorsal gut, postanal ventral, 1 or 2 hypural on body, ventral gut just anterior to anus and further anterior, eight have crown and nape, one has crown only and one has neither, two have lower jar tip.
S. variegatus	29	All have dorsal gut, postanal ventral, 1 or 2 hypural on body, ventral gut just anterior to anus, eight have crown, four have nape, eight have dorsal notochord tip, three have lower jar tip.
S. zacentrus	11	All have dorsal gut, postanal ventral (shorter row than in most other species), ventral gut just anterior to anus, two have ventral gut slightly anterior also, one has crown, one has nape, two have hypural in finfold, one has considerable end of notochord, one has hypural on body, one has lower jaw tip.

Table 3. Species and subgenera of rockfish (*Sebastes* spp.) used in mitochondrial DNA haplotype comparisons.

Common Name	Species	Subgenus
Pacific ocean perch	*Sebastes alutus*	*Acutomentum*
rosethorn rockfish	*Sebastes helvomaculatus*	*Sebastomus*
quillback rockfish	*Sebastes maliger*	*Pteropodus*
redbanded rockfish	*Sebastes babcocki*	*Rosicola*
black rockfish	*Sebastes melanops*	*Sebastosomus*
yellowtail rockfish	*Sebastes flavidus*	*Sebastosomus*
sharpchin rockfish	*Sebastes zacentrus*	*Allosebastes*
harlequin rockfish	*Sebastes variegatus*	*Allosebastes*
redstripe rockfish	*Sebastes proriger*	*Allosebastes*
rougheye rockfish	*Sebastes aleutianus*	*Zalopyr*
yelloweye rockfish	*Sebastes ruberrimus*	*Sebastopyr*
shortraker rockfish	*Sebastes borealis*	*Zalopyr*
light dusky rockfish	*Sebastes ciliatus*	*Sebastosomus*
silvergray rockfish	*Sebastes brevispinis*	*Acutomentum*
copper rockfish	*Sebastes caurinus*	*Pteropodus*

Table 4. Restriction enzyme fragment patterns of larval and reference adult rockfish for which the patterns correspond. Letters are haplotypes referenced in the Appendix. "-" means the haplotype differs by 1 site loss, and "+" means that the haplotype differs by 1 site gain.

Bst N I fragment sizes

S. ruberrimus	*S. babcocki* *S. maliger* *S. proriger* *S. variegatus* *S. zacentrus* *S. helvomaculatus* *S. aleutianus* *S. borealis* *S. caurinus*			*S. brevispinis*
C	F	F+	F-	G
1102	717	1111	865	717
631	631	717	717	480
480	480	385	631	385
112	385	112	112	343
60	112	60	60	288
	60			112
				60

Dde I fragment sizes

S. ruberrimus		*S. brevispinis*		*S. proriger* *S. variegatus* *S. zacentrus*			*S. melanops* *S. babcocki*		*S. caurinus* *S. maliger*
B	B+	C	D	E	E+a	E+b	F	F+	L
1322	1189	977	1011	1011	908	1011	1011	1011	806
462	462	462	462	462	462	462	462	462	462
298	298	336	311	311	311	216	254	254	254
216	216	216	216	216	216	195	195	195	195
38	133	178	195	195	195	178	194	164	178
38	38	133	103	103	103	133	117	117	117
11	38	38	38	38	103	103	103	103	105
	11	34	11	38	38	38	38	38	71
		11		11	38	38	11	30	59
					11	11		11	41
									38
									32
									16
									11

Mbo I fragment sizes

	S. caurinus *S. maliger*		*S. variegatus* *S. zacentrus*		*S. babcocki*		*S. ruberrimus*		*S. aleutianus* *S. brevispinis* *S. proriger*
	C		D		G		I		K
707	707	707	707	594	594	560	560	594	594
560	560	450	450	560	560	505	505	560	560
406	406	406	406	445	445	406	406	406	406
332	332	332	332	406	406	332	332	332	332
198	198	198	198	198	198	198	198	198	198
182	182	182	182	103	103	182	182	182	182
2385		110	110	79	79	113	113	113	113
						89	89		

Table 4. Continued

Msp I fragment sizes		
S. alutus *S. helvomaculatus* *S. babcocki* *S. melanops* *S. flavidus* *S. zacentrus* *S. variegatus* *S. borealis* *S. ruberrimus* *S. ciliatus*	*S. caurinus* *S. maliger*	
B	C	
1708	1708	
284	372	
275	275	
88	30	
30		

Rsa I fragment sizes			
S. borealis *S. brevispinis* *S. caurinus* *S. brevispinis* *S. babcocki* *S. maliger* *S. proriger* *S. variegatus*		*S. alutus* *S. helvomaculatus* *S. zacentrus*	*S. aleutianus* *S. ruberrimus*
B	B-	C	D
1985	2385	1863	1639
400		400	400
		122	346

Table 5. Comparison of larvae in various pigment groups and those identified genetically.

	Numbers of larvae		Numbers of genetic identifications							
Pigment group	Pigment groups	Genetic ids.	?	*S. babcocki*	*S. brevispinus*	*S. maliger/ caurinus*	*S. proriger*	*S. ruberrimus*	*S. variegatus*	*S. zacentrus*
1	5	4				4				
2	18	10					1		8	1
3	10	7		1	1			2	2	1
4	14	10		1				7	2	
5	7	6	1						4	1
6	4	3					3			
7	37	21			3		1	2	13	2
8	8	6								6
Totals	103	67	1	2	4	4	5	11	29	11

Appendix

Appendix. pigment characters, pigment groups and genetic identifications of *Sebastes* larvae from *Cobb* cruise 9809.

		Pigment loci															Pigment	Genetic id.
Tow	Fish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	group	*Sebastes*
22	6		1	1	1		1	1			1	1	1				1	genetics not analyzed
2	1		1	1	1		1	1	1	1	1	1	1				1	*maliger/caurinus*
8	3	1	1	1	1			1		1	1	1	1				1	*maliger/caurinus*
22	1			1	1		1	1	1	1	1	1	1				1	*maliger/caurinus*
25	3	1	1	1	1		1	1	1	1	1	1	1				1	*maliger/caurinus*
2	6		1	1		1			1	1	1		1				2	genetics not analyzed
8	5					1				1	1		1	1			2	genetics not analyzed
19	4					1			1	1	1		1		1	1	2	genetics not analyzed
20	8			1		1				1	1		1		1		2	genetics not analyzed
21	3					1				1	1		1		1	1	2	genetics not analyzed
22	3					1			1		1		1	1	1	1	2	genetics not analyzed
23	10			1		1			1	1	1		1		1		2	genetics not analyzed
25	2					1			1	1	1		1		1	1	2	genetics not analyzed
23	16		1			1			1	1	1		1		1	1	2	*proriger*
20	10		1	1		1					1		1		1		2	*variegatus*
20	14	1	1	1		1				1	1		1		1	1	2	*variegatus*
21	10		1			1			1		1		1		1	1	2	*variegatus*
22	4					1			1		1		1		1	1	2	*variegatus*
23	9					1			1	1	1		1	1	1	1	2	*variegatus*
23	17					1			1		1		1		1	1	2	*variegatus*
16	1					1				1	1		1		1		2	*variegatus*
19	5					1			1	1	1		1	1	1		2	*variegatus*
23	4	1				1					1		1		1	1	2	*zacentrus*
8	1	1		1				1	1	1	1		1	1	1	1	3	*babcocki*
18	3	1						1	1	1	1		1		1	1	3	*brevispinus*
7	2	1	1	1						1	1		1	1	1		3	genetics not analyzed
20	4	1	1						1		1		1		1		3	genetics not analyzed
20	11	1							1	1	1		1		1		3	genetics not analyzed
22	2	1	1	1					1	1	1		1		1	1	3	*ruberrimus*
24	1	1	1						1		1		1		1	1	3	*ruberrimus*
2	7	1								1	1		1		1	1	3	*variegatus*
22	7	1		1				1			1		1		1	1	3	*variegatus*
25	1	1		1						1	1		1				3	*zacentrus*
4	1		1	1							1		1		1	1	4	*babcocki*
2	4		1	1						1	1		1		1	1	4	genetics not analyzed
3	1		1	1						1	1		1		1	1	4	genetics not analyzed
6	2		1	1						1	1		1		1	1	4	genetics not analyzed
26	1		1	1					1	1	1		1		1		4	genetics not analyzed
18	2		1	1					1	1	1		1		1		4	*ruberrimus*
20	1		1	1				1	1		1		1		1	1	4	*ruberrimus*
20	3		1	1					1	1	1		1		1	1	4	*ruberrimus*
23	7		1	1					1	1	1		1		1	1	4	*ruberrimus*
23	14		1	1					1	1	1		1		1		4	*ruberrimus*
24	2		1	1					1	1	1		1		1	1	4	*ruberrimus*
25	5		1	1					1		1		1		1		4	*ruberrimus*
5	1		1	1						1	1		1		1		4	*variegatus*
21	4		1	1					1	1	1		1		1	1	4	*variegatus*
1	1		1							1	1		1	1	1	1	5	?
21	5		1							1	1		1		1	1	5	genetics not analyzed
2	5		1							1	1		1	1			5	*variegatus*
4	2		1						1		1		1		1		5	*variegatus*

Appendix. pigment characters, pigment groups and genetic identifications of *Sebastes* larvae from *Cobb* cruise 9809.

		Pigment loci															Pigment	Genetic id.
Tow	Fish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	group	*Sebastes*
19	6		1						1	1	1		1		1	1	5	*variegatus*
23	5		1							1	1		1		1		5	*variegatus*
20	2		1					1	1		1		1			1	5	*zacentrus*
25	4			1						1	1		1		1		6	genetics not analyzed
7	4			1					1	1	1		1		1		6	*proriger*
14	3			1						1	1		1		1		6	*proriger*
21	2			1						1	1		1		1		6	*proriger*
15	2								1	1	1		1		1	1	7	*brevispinus*
16	2								1		1		1		1	1	7	*brevispinus*
21	7									1	1		1		1	1	7	*brevispinus*
1	2									1	1		1		1		7	genetics not analyzed
14	1								1	1	1		1	1	1	1	7	genetics not analyzed
14	4									1	1		1	1	1		7	genetics not analyzed
16	3								1	1	1		1		1		7	genetics not analyzed
18	1								1	1	1		1	1	1		7	genetics not analyzed
18	4								1	1	1		1		1		7	genetics not analyzed
19	1									1	1		1		1		7	genetics not analyzed
19	2									1	1		1	1	1		7	genetics not analyzed
20	5								1	1	1		1		1		7	genetics not analyzed
21	6									1	1		1		1		7	genetics not analyzed
21	9								1		1		1		1	1	7	genetics not analyzed
22	5									1	1		1		1	1	7	genetics not analyzed
23	1								1		1		1		1	1	7	genetics not analyzed
23	2								1	1	1		1		1		7	genetics not analyzed
23	11									1	1		1		1	1	7	genetics not analyzed
23	15										1		1		1	1	7	genetics not analyzed
17	1										1		1	1	1	1	7	*proriger*
20	9							1	1	1	1		1	1	1		7	*ruberrimus*
20	13								1	1	1		1		1	1	7	*ruberrimus*
2	2									1	1		1		1		7	*variegatus*
8	2									1	1		1		1		7	*variegatus*
8	6								1	1	1		1		1		7	*variegatus*
14	2									1	1		1	1	1		7	*variegatus*
15	1									1	1		1		1		7	*variegatus*
16	4								1		1		1		1	1	7	*variegatus*
16	5								1	1	1		1	1	1		7	*variegatus*
20	12									1	1		1		1		7	*variegatus*
21	**1**									**1**	**1**		**1**		**1**	**1**	**7**	***variegatus***
21	8								1		1		1		1	1	7	*variegatus*
23	8								1		1		1	1	1		7	*variegatus*
23	12									1	1		1		1		7	*variegatus*
23	13									1	1		1		1		7	*variegatus*
23	3								1		1		1			1	7	*zacentrus*
23	6									1	1		1	1			7	*zacentrus*
16	6								1		1		1				8	genetics not analyzed
20	6								1		1		1				8	genetics not analyzed
3	2									1	1		1				8	*zacentrus*
7	1									1	1		1				8	*zacentrus*
7	3									1	1		1				8	*zacentrus*
8	4								1	1	1		1				8	*zacentrus*

Appendix. pigment characters, pigment groups and genetic identifications of *Sebastes* larvae from *Cobb* cruise 9809.

		Pigment loci															Pigment	Genetic id.
Tow	Fish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	group	*Sebastes*
19	3								1		1		1				8	*zacentrus*

Appendix. Composite haplotypes, pigment characters, pigment groups and genetic identifications of *Sebastes* larvae from *Cobb* cruise 9809.
Keys for the haplotypes are in Table 4.

Tow	Fish	Composite haplotypes					Pigment loci														Pigment	Genetic identification
		*Bst*NI	*Dde* I	*Mbo* I	*Rsa* I	*Msp* I	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	group	*Sebastes*
22	6	C	B	I	D			1	1	1		1	1			1	1	1				1	genetics not analyzed
2	1	F	L	C	B			1	1	1		1	1	1	1	1	1	1				1	*maliger/caurinus*
8	3	F	L	C	B		1	1	1	1			1		1	1	1	1				1	*maliger/caurinus*
22	1								1	1		1	1	1	1	1	1	1				1	*maliger/caurinus*
25	3	F	L	C	B-		1	1	1	1		1	1	1	1	1	1	1				1	*maliger/caurinus*
2	6							1	1		1			1	1	1		1				2	genetics not analyzed
8	5										1				1	1		1	1			2	genetics not analyzed
19	4										1			1	1	1		1		1	1	2	genetics not analyzed
20	8								1		1				1	1		1		1		2	genetics not analyzed
21	3										1				1	1		1		1	1	2	genetics not analyzed
22	3										1			1		1		1	1	1	1	2	genetics not analyzed
23	10								1		1			1	1	1		1		1		2	genetics not analyzed
25	2										1			1	1	1		1		1	1	2	genetics not analyzed
23	16	F	E	D	B			1			1			1	1	1		1		1	1	2	*proriger*
20	10	F	E	D	B			1	1		1					1		1		1		2	*variegatus*
20	14						1	1	1		1				1	1		1		1	1	2	*variegatus*
21	10	F	E	D	B			1			1			1		1		1		1	1	2	*variegatus*
22	4										1			1		1		1		1	1	2	*variegatus*
23	9	F	E	D	B						1			1	1	1		1	1	1	1	2	*variegatus*
23	17										1			1		1		1		1	1	2	*variegatus*
16	1	F	E	D	B						1				1	1		1		1		2	*variegatus*
19	5	F+	E+a	D	B	B					1			1	1	1		1	1	1		2	*variegatus*
23	4	F	E	D	C		1				1					1		1		1	1	2	*zacentrus*
8	1	F	F+	G	B	B	1		1				1	1	1	1		1	1	1	1	3	*babcocki*
18	3	G	C	K	B		1						1	1	1	1		1		1	1	3	*brevispinus*
7	2						1	1	1						1	1		1	1	1		3	genetics not analyzed
20	4						1	1						1		1		1		1		3	genetics not analyzed
20	11	F	E	D	B		1							1	1	1		1		1		3	genetics not analyzed
22	2						1	1	1					1	1	1		1		1	1	3	*ruberrimus*
24	1	C	B	I	D		1	1						1		1		1		1	1	3	*ruberrimus*
2	7	F	E+b	D	B	B	1								1	1		1		1	1	3	*variegatus*
22	7	F	E	D	B		1		1				1			1		1		1	1	3	*variegatus*
25	1	F	E	D	C		1		1						1	1		1				3	*zacentrus*
4	1	F	F+	G	B	B		1	1							1		1		1	1	4	*babcocki*
2	4							1	1						1	1		1		1	1	4	genetics not analyzed
3	1							1	1						1	1		1		1	1	4	genetics not analyzed
6	2							1	1						1	1		1		1	1	4	genetics not analyzed
26	1							1	1					1	1	1		1		1		4	genetics not analyzed
18	2	C	B	I	D			1	1					1	1	1		1		1		4	*ruberrimus*
20	1	C	B	I	D			1	1				1	1		1		1		1	1	4	*ruberrimus*
20	3	C	B	I	D			1	1					1	1	1		1		1	1	4	*ruberrimus*
23	7	C	B	I	D			1	1					1	1	1		1		1	1	4	*ruberrimus*
23	14	C	B	I	D			1	1					1	1	1		1		1		4	*ruberrimus*
24	2	C	B+	I	D	B		1	1					1	1	1		1		1	1	4	*ruberrimus*
25	5	C	B	I	D			1	1					1		1		1		1		4	*ruberrimus*
5	1	F	E	D	B			1	1						1	1		1		1		4	*variegatus*
21	4	F	E	D	B			1	1					1	1	1		1		1	1	4	*variegatus*
1	1	F	D	D	C	C		1							1	1		1	1	1	1	5	*?*
21	5							1							1	1		1		1	1	5	genetics not analyzed
2	5	F+	E	D	B	B		1							1	1		1	1			5	*variegatus*
4	2	F	E	D	B			1						1		1		1		1		5	*variegatus*
19	6	F	E	D	B			1						1	1	1		1		1	1	5	*variegatus*
23	5	F	E	D	B			1							1	1		1		1		5	*variegatus*
20	2	F	E	D	C			1					1	1		1		1			1	5	*zacentrus*
25	4								1						1	1		1		1		6	genetics not analyzed
7	4	F	E	K	B				1					1	1	1		1		1		6	*proriger*
14	3	F	E	K	B				1						1	1		1		1		6	*proriger*
21	2	F	E	K	B				1						1	1		1		1		6	*proriger*
15	2	G	C	K	B									1	1	1		1		1	1	7	*brevispinus*
16	2	G	C	K	B									1		1		1		1	1	7	*brevispinus*
21	7	G	C	K	B										1	1		1		1	1	7	*brevispinus*
1	2														1	1		1		1		7	genetics not analyzed
14	1													1	1	1		1	1	1	1	7	genetics not analyzed
14	4														1	1		1	1	1		7	genetics not analyzed
16	3													1	1	1		1		1		7	genetics not analyzed
18	1													1	1	1		1	1	1		7	genetics not analyzed
18	4													1	1	1		1		1		7	genetics not analyzed

Tow	Fish	Composite haplotypes					Pigment loci														Pigment	Genetic identification
		*Bst*N I	*Dde* I	*Mbo* I	*Rsa* I	*Msp* I	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	group	*Sebastes*
Appendix continued																							
19	1														1	1		1		1		7	genetics not analyzed
19	2														1	1		1	1	1		7	genetics not analyzed
20	5													1	1	1		1		1		7	genetics not analyzed
21	6														1	1		1		1		7	genetics not analyzed
21	9													1		1		1		1	1	7	genetics not analyzed
22	5 F		L		C	B									1	1		1		1	1	7	genetics not analyzed
23	1 F		E		D	B								1		1		1		1	1	7	genetics not analyzed
23	2													1	1	1		1		1		7	genetics not analyzed
23	11														1	1		1		1	1	7	genetics not analyzed
23	15 F		E		K	B										1		1		1	1	7	genetics not analyzed
17	1 F		E		K	B										1		1	1	1	1	7	*proriger*
20	9 C		B		I	D							1	1	1	1		1	1	1		7	*ruberrimus*
20	13 F		E		D	B								1	1	1		1		1	1	7	*ruberrimus*
2	2 F		E		D	B									1	1		1		1		7	*variegatus*
8	2 F		E		D	B									1	1		1		1		7	*variegatus*
8	6 F		E		D	B								1	1	1		1		1		7	*variegatus*
14	2 F		E		D	B									1	1		1	1	1		7	*variegatus*
15	1 F		E		D	B									1	1		1		1		7	*variegatus*
16	4 F		E		D	B								1		1		1		1	1	7	*variegatus*
16	5 F		E+a		D	B	B							1	1	1		1	1	1		7	*variegatus*
20	12 C		B		I	D									1	1		1		1		7	*variegatus*
21	1 F		E		D	B									1	1		1		1	1	7	*variegatus*
21	8 F		E		D	B								1		1		1		1	1	7	*variegatus*
23	8 F		E		D	B								1		1		1	1	1		7	*variegatus*
23	12 F		E		D	B									1	1		1		1		7	*variegatus*
23	13 F		E+b		D	B	B								1	1		1		1		7	*variegatus*
23	3 F		E		D	C								1		1		1			1	7	*zacentrus*
23	6 F		E		D	C									1	1		1	1			7	*zacentrus*
16	6													1		1		1				8	genetics not analyzed
20	6													1		1		1				8	genetics not analyzed
3	2 F		E		D	C									1	1		1				8	*zacentrus*
7	1 F		E		D	C									1	1		1				8	*zacentrus*
7	3 F		E+b		D	C	B								1	1		1				8	*zacentrus*
8	4 F		E		D	C								1	1	1		1				8	*zacentrus*
19	3 F		E		D	C								1		1		1				8	*zacentrus*
20	7 F		E		D	C								1		1		1				8	*zacentrus*
18	6 G		C		K	B							pigment not determined										*brevispinis*
8	13 F		E		K	B							pigment not determined										*proriger*
10	3 F		E		K	B							pigment not determined										*proriger*
8	17 C		B		I	D							pigment not determined										*ruberrimus*
9	9 C		B		I	D							pigment not determined										*ruberrimus*
8	14 F		E		D	B							pigment not determined										*variegatus*
9	5 F		E		D	B							pigment not determined										*variegatus*
9	10 F		E		D	B							pigment not determined										*variegatus*
10	2 F		E		D	B							pigment not determined										*variegatus*
10	6 F		E		D	B							pigment not determined										*variegatus*
13	1 F		E		D	B							pigment not determined										*variegatus*
18	5 F-		E		D	B	B						pigment not determined										*variegatus*
18	8 F		E		D	B							pigment not determined										*variegatus*
8	7 F		E		D	C							pigment not determined										*zacentrus*
8	9 F		E		D	C							pigment not determined										*zacentrus*
9	1 F		E		D	C							pigment not determined										*zacentrus*
13	2 F		E		D	C							pigment not determined										*zacentrus*