New Data on Distribution and Biology of Gray, Angry, and Northern Rockfishes from the Northwestern Pacific

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Abstract  
Despite relatively small numbers of rockfish species inhabiting the western North Pacific and quite intensive studies conducted recently in the Pacific waters off the northern Kuril Islands and southeastern Kamchatka, the distribution patterns and biological features of gray rockfish *Sebastes glaucus*, angry rockfish *S. iracundus*, and northern rockfish *S. polyspinis*, remain poorly understood. On the basis of data collected during 1993-2000 bottom trawl surveys and 1992-2002 commercial fishing operations within the depth range of 83-850 m, some quantitative indices of occurrence of gray, angry, and northern rockfishes in the study area are provided. In addition, their spatial and depth distributions are analyzed, bottom temperature preferences are shown, size composition and length-weight relationships are considered, and the diurnal, seasonal, and multi-annual changes of catch rates and occurrence in catches are analyzed.

Introduction  
Rockfishes (Sebastidae) in Pacific waters off the northern Kuril Islands and southeastern Kamchatka, with at least 12-13 species, are less diverse than in the northeastern Pacific (Fedorov 2000, Sheiko and Fedorov 2000). Many of them are common or abundant and several
species, such as Pacific ocean perch *Sebastes alutus*, shortraker rockfish *S. borealis*, rougheye rockfish *S. aleutianus*, broadbanded thornyhead *Sebastolobus macrochir*, and shortspine thornyhead *S. alascanus* are commercially important targets of bottom trawl, longline, and gillnet fisheries. The distribution and biology of these species in the study area are rather well-studied. Despite quite intensive research conducted recently in the Pacific waters off the northern Kuril Islands and southeastern Kamchatka the distribution patterns and biological features of three rockfish species (gray rockfish *Sebastes glaucus*, angry rockfish *S. iracundus*, and northern rockfish *S. polyspinis*) remain poorly understood. The angry rockfish is believed to be a temporary migrant from southern areas (Orlov et al. 1998), northern rockfish recently penetrated the study area from the neighboring Aleutian Islands waters (Orlov 2004), while the status of gray rockfish is still uncertain. Until now, most publications dealing with these species in the northwestern Pacific contain data on their taxonomy or general information regarding zoogeography, depth ranges, and maximum size (Moiseev 1937; Snytko and Fedorov 1974; Barsukov 1981, 2003; Masuda et al. 1984; Lindberg and Krasyukova 1987; Amaoka et al. 1995; Fedorov and Parin 1998; Borets 2000; Fedorov 2000; Sheiko and Fedorov 2000; Snytko 2001; Chereshnev et al. 2001; Novikov et al. 2002; Parin et al. 2002; Fedorov et al. 2003).

The main purpose of this paper is (1) to provide some quantitative indices of the occurrence of three insufficiently studied rockfish species in Pacific waters off the northern Kuril Islands and southeastern Kamchatka, (2) to consider patterns of their spatial and depth distributions, (3) to analyze their catch rates and occurrence depending on bottom temperature, time of the day, season, and between years, and (4) to show their size compositions and relations between length and body weight.

**Materials and methods**

This paper is based on materials sampled in the framework of the scientific program for species that had not been studied adequately, or were not being sufficiently utilized on the continental slope of the Russian Far Eastern seas. This paper presents the results of analysis of catches from about 1,500 bottom trawl survey hauls (19 bottom trawl surveys, 1993-2000) and over 10,000 commercial fishing operations (about 50 cruises, March-December 1992-2002) in Pacific waters off the northern Kuril Islands and southeastern Kamchatka (between 47°50’ and 52°00’N, depths 83-850 m). Positions of bottom trawl stations were randomly selected. Commercial fishing efforts varied by depth, season, and areas depending on targeted species. However, since trawlers fished for a variety of species (from shallow-water flatfishes and Pacific cod to deepwater rockfishes, thornyheads, and sablefish), the whole study
area was covered by a dense grid of trawlings, and between-year maps of distribution of survey stations and commercial trawlings were quite similar (Orlov 2003). These research cruises were conducted jointly by the Russian Federal Research Institute of Fisheries and Oceanography (VNIRO) with Kamchatka (KamchatNIRO) and Sakhalin (SakhNIRO) where the distribution and biology data on three rockfish species were collected. That study was done aboard the Japanese trawlers (Tomi-Maru 53, Tomi-Maru 82 and Tora-Maru 58) specially equipped for ground hauls on the parts of the continental slope having rough relief. The bottom temperature was measured during most of the hauls. Trawls were made over 24 hours, with the bottom trawls having vertical (5-7 m) and horizontal (25 m) openings and 100 mm mesh size in codend. The trawl opening parameters were monitored instrumentally and the mean speed was 3.6 knots. Since the duration of hauls during the cruises varied between 0.5 and 10 hours, all catches were subsequently recalculated into standard 1 hour hauls. The distribution of individual species by depth and bottom temperature was analyzed according to the percentage of their occurrence calculated by average 1 hour haul catches. Analysis of species occurrence, spatial and vertical distributions, bottom temperature preferences, composition of species co-occurring, diurnal, seasonal and multi-annual changes of catch rates, and frequency of occurrence are based on 251 captures of gray rockfish, 32 captures of angry rockfish, and 77 captures of northern rockfish. Length frequency distributions are based on measurements of 282 specimens of gray rockfish, 119 specimens of angry rockfish, and 162 specimens of northern rockfish. Length-weight relations are analyzed using measurements and weights of 281 gray rockfish, 38 angry rockfish, and 88 northern rockfish. The relation between fish fork length (FL, cm) and weight (W, g) is described by the following equation: $W = aFL^b$, where $a$ and $b$ are linear and exponential coefficients respectively. Limited data (62 specimens of gray rockfish, 31 specimens of angry rockfish, and 27 specimens of northern rockfish examined) were analyzed to obtain some information about sex ratios, size differences between males and females, and stomach fullness. Stomach fullness index (SFI) was determined on a five number scale (0 = empty, 4 = full).

Results and discussion

**General information and occurrence in the study area**

**Gray rockfish**

The gray rockfish is distributed in the North Pacific mostly within Asian waters (Sea of Japan, Sea of Okhotsk, Bering Sea, Pacific waters off Japan, Kuril Islands, and Kamchatka). Its range is limited in the north by the Bering Sea while in the south the species reaches Japanese waters off Iwate Prefecture (northern Honshu). In the Sea of Japan off the conti-
nental coast, the gray rockfish is distributed northward to Tatar Strait and to Peter the Great Bay southward; off the Japanese coast it reaches Toyama Bay in the south (Masuda et al. 1984, Snytko 2001). Recently this rockfish was found off Attu Island, in the Aleutian Islands (Orr and Baker 1996). The abundance of gray rockfish in the majority of areas of its range is rather low. It is most abundant in the northern Sea of Okhotsk where it is a commercially important fishery target (Chereshnev et al. 2001, Fedorov et al. 2003). Its biology is little known (Novikov et al. 2002, Love et al. 2002). The gray rockfish probably plays a certain role in trophic webs being consumed by fin whales (Barsukov 2003). The data on life history of the gray rockfish to the present were limited to waters off Kamchatka, northern and southwestern Sea of Okhotsk, where this species is considered a prospective target of longline and bottom trawl inshore fisheries (Panchenko 1996, Kondratiev 1996, Chetvergov 1998, Nemchinov 2001).

In the study area, gray rockfish is believed to be abundant (Fedorov and Parin 1998, Sheiko and Fedorov 2000). Among the three rockfishes this species was taken most frequently (251 captures). However, while it was caught most frequently (Table 1) the average catch rate was the lowest of the three species. This may be evidence of the fact that gray rockfish in some periods is able to form dense concentrations. However, during most of the year this species is very scarce in the study area and is caught only occasionally. In addition, during summer months this species in Kamchatkan waters primarily inhabits inner shelf waters (Chetvergov 1998). Since the studies were limited to depths no shallower then 80 m, our observation based on bottom trawl catches probably provides an underestimate of its real abundance in the study area.

**Angry rockfish**

At present, there is no common point of view regarding the taxonomic status of the angry rockfish (Barsukov 2003). The majority of authors suggest the existence in the northwestern Pacific of two congeneric rockfish species, *Sebastes flammeus* and *S. iracundus* (Snytko and Fedorov 1974, Masuda et al. 1984, Nagasawa and Torisawa 1991, Amaoka et al. 1995) differing in size, depth range, and spawning grounds. Other researchers (Barsukov 1981, Snytko 2001) consider them subspecies, *Sebastes iracundus iracundus* and *Sebastes iracundus flammeus*. The information regarding their range is also very controversial. The angry rockfish is distributed from the Pacific coast of Japan (Chiba Prefecture) along the Kuril Islands to Emperor Seamounts (Masuda et al. 1984), in the Sea of Japan off Moneron Island and in the southwestern Sea of Okhotsk (Snytko and Fedorov 1974, Romanov 1999) and off Tennou seamount (Nagasawa and Torisawa 1991). Snytko (2001) states that this species also inhabits waters of southeastern Kamchatka, the Commander Islands, and the Aleutians as far as Amchitka Strait and Bowers Ridge in
### Table 1. Some quantitative indices of occurrence of gray, angry, and northern rockfishes in catches within the Pacific waters off the northern Kuril Islands and southeastern Kamchatka, 1992-2002.

<table>
<thead>
<tr>
<th>Species</th>
<th>Proportion in catches %</th>
<th>Number of fish caught</th>
<th>Weight of fish caught</th>
<th>Depth, m</th>
<th>Bottom temperature, °C</th>
<th>Length, cm</th>
<th>Weight, g</th>
<th>Number of hauls with species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray rockfish</td>
<td>0.001-9.43</td>
<td>1-944</td>
<td>0.251</td>
<td>83-390</td>
<td>−1.0-3.5</td>
<td>19.57</td>
<td>140-3300</td>
<td>251</td>
</tr>
<tr>
<td></td>
<td>0.176</td>
<td>10.15</td>
<td>3.66</td>
<td>20.68</td>
<td>5.54</td>
<td>185.6</td>
<td>1.40</td>
<td>44.78</td>
</tr>
<tr>
<td>Angry rockfish</td>
<td>0.008-5.57</td>
<td>1-210</td>
<td>0.38</td>
<td>1-462</td>
<td>0.84</td>
<td>370-650</td>
<td>3.1-3.5</td>
<td>26.67</td>
</tr>
<tr>
<td></td>
<td>1.163</td>
<td>24.15</td>
<td>4.61</td>
<td>44.97</td>
<td>8.44</td>
<td>473.4</td>
<td>3.41</td>
<td>46.55</td>
</tr>
<tr>
<td>Northern rockfish</td>
<td>0.8-7.69</td>
<td>1-524</td>
<td>0.123</td>
<td>0.90</td>
<td>0.67</td>
<td>101-466</td>
<td>209.4</td>
<td>5.90</td>
</tr>
<tr>
<td></td>
<td>0.212</td>
<td>18.70</td>
<td>7.69</td>
<td>3.59</td>
<td>5.90</td>
<td>190.4</td>
<td>1.91</td>
<td>32.38</td>
</tr>
</tbody>
</table>

Note: above the line minimum and maximum values are given, while under the line average values are provided.
the Bering Sea. However, some authors (Sheiko and Fedorov 2000) doubt whether angry rockfish live in Kamchatkan waters. It is most probable that the suggestion is correct and angry rockfish does not inhabit waters off Kamchatka and the northern East Pacific, because it was not included in recent taxonomic reviews of Alaska and northeastern Pacific rockfishes (Kramer and O’Connell 1995, Orr et al. 2000, Love et al. 2002, Mecklenburg et al. 2002).

The flesh of angry rockfish is very delicious (Snytko 2001); it has high demand at fish markets in Japan where it is used for cooking traditional Japanese dishes such as sushi and sashimi and for frying as well. Angry rockfish probably plays a certain role in food webs since it is the most abundant rockfish species in the Pacific waters off Japan (Nagasawa and Torisawa 1991), and is known to be eaten by sperm whales (Barsukov 2003). The publications dealing with this species contain some data on its morphology (Sasaki 1976, Romanov 1999) and general information on life history aspects in Japanese waters (Nagasawa and Torisawa 1991).

In Pacific waters off the northern Kuril Islands, the angry rockfish was rarest among the species considered (32 captures) though the data provided are probably understated due to difficulties of species identification. In catches angry rockfish co-occurred most frequently with the similar-appearing shortraker rockfish (Barsukov 1981).

**Northern rockfish**

The northern rockfish inhabits primarily North American waters from British Columbia (Graham Island) in the south to the eastern Bering Sea (to 60°N) in the north, including the Gulf of Alaska and waters off the Aleutian Islands (Allen and Smith 1988, Snytko 2001). In Asian waters, this species is known from occasional captures in the western Bering Sea, off southeastern Kamchatka and the northern Kuril Islands as far as Onekotan Island in the south (Snytko and Fedorov 1974, Fedorov and Parin 1998, Snytko 2001, Parin et al. 2002). The northern rockfish is believed to be one of the most abundant rockfishes of the North Pacific (Moiseev and Parakketsov 1961). It is most abundant in the Gulf of Alaska, off the Aleutian Islands and in the eastern Bering Sea (Harrison 1993, Ronholt et al. 1994, Martin 1997, Snytko 2001) and off the Commander Islands as well (Mecklenburg et al. 2002). The species is very important in the U.S. bottom trawl fisheries in the Gulf of Alaska and off the Aleutians as an export to Japan and Korea (Love et al. 2002, Clausen and Heifetz 2002). The majority of published data on the biology of the northern rockfish comes from the northeastern Pacific (Westrheim and Tsuyuki 1971; Mito 1974; Brodeur and Livingston 1988; Yang 1993, 1996; Cailliet et al. 2001; Clausen and Heifetz 2002). The life cycle of this species in Asian waters is little known. Until the present, there were only few data on its occurrence and some biological characters in the
western Bering Sea and Pacific waters off the northern Kuril Islands and southeastern Kamchatka (Moiseev and Paraketsov 1961; Skalkin 1964; Orlov 2000, 2001).

Some authors (Sheiko and Fedorov 2000, Parin et al. 2002) consider the northern rockfish in the study area to be abundant while others (Fedorov and Parin 1998) suggest it is rare. Our results show that the northern rockfish was rather rare in the study area (77 captures). Its maximum catches exceeded 500 individuals per haul (mean 18.7 individuals), with an average of 7.7 specimens or 3.6 kg per hour of trawling. These data show that northern rockfish catch rates are very low for most of the year, but this species may form increased concentrations during some periods.

**Spatial distribution**

The gray rockfish in the study area was most abundant opposite the First Kuril Strait and off the western slope of the underwater plateau in the southern part of the area surveyed (Fig. 1a), where catches exceeded 250 kg per hour trawling. The majority of captures occurred north of the Forth Kuril Strait though most catches had several specimens only. The existence of dense concentrations opposite straits suggests that the majority of gray rockfish are able to migrate to the study area from the Sea of Okhotsk, mostly through shallow First and Second Kuril Straits. However, it will be further shown that the gray rockfish inhabits this area permanently. We speculate that the relatively dense concentrations opposite straits are probably related to better feeding conditions within these areas due to higher water exchange with the Sea of Okhotsk. The persistence of dense concentrations off the underwater plateau, where the quasi-stationary eddy exists, may be evidence of transport of pelagic juveniles here from the north and of their further dwelling within eddy waters until settlement. In the Sea of Japan and southwestern Sea of Okhotsk, the gray rockfish inhabits sludgy and sandy grounds (Lindberg and Krasyukova 1987, Panchenko 1996, Novikov et al. 2002). However, Chetvergov (1998) pointed out that this species in the waters off Kamchatka and the Commander Islands preferred areas with complex bottom relief. Apparently, in this study area, gray rockfish exhibits more plasticity regarding bottom type preference, since it forms dense concentrations on rather soft grounds north of the Forth Kuril Strait and off the underwater plateau which is characterized by rocky relief and rough bottom.

The angry rockfish occurred exclusively within the southern part of the study area (Fig. 1b). At the same time, almost all captures were off the eastern slope of the underwater plateau; only a single record was observed outside this area, opposite Onekotan Island. The data obtained confirm previous suggestions that this species migrates to the northern Kurils from southern areas (Orlov et al. 1998), and also
Figure 1. Spatial distribution and relative abundance of (a) gray, (b) angry, and (c) northern rockfishes in Pacific waters off the northern Kuril Islands and southeastern Kamchatka, 1992-2002.
that no angry rockfish inhabit Kamchatkan waters (Sheiko and Fedorov 2000). There are no data on spatial distribution of species considered in other areas.

The northern rockfish occurred within the entire area surveyed from 48°N in the south to 52°N in the north (Fig. 1a), though its maximum catches (over 25 kg per hour trawling) were registered in the southeastern part of the study site. Previous to the mid 1990s, northern rockfish were not captured in this area. It was in May 1996, that a few fish (spawning females) were first captured. It is possible that the northern rockfish migrated to the Kurils and Kamchatka from the Aleutians (Orlov 2004) and found favorable conditions for spawning within the southern part of the study area, where a quasi-stationary eddy exists (Orlov 2003). In waters off the Aleutian Islands and in the western Gulf of Alaska, northern rockfish are also most abundant off seamounts where they prefer hard and rough bottoms (Ronholt et al. 1994, Martin 1997, Clausen and Heifetz 2002).

**Bathymetry**

According to recent data, the gray rockfish within its range occupies depths of 2-550 m (Orr et al. 2000; Love et al. 2002) though some authors believe that the maximum depth of its occurrence is 370 m with
Figure 2. Vertical distribution of (a) gray, (b) angry, and (c) northern rockfishes in Pacific waters off the northern Kuril Islands and southeastern Kamchatka, 1992-2002.
an optimum at 20-40 m (Fedorov 2000, Sheiko and Fedorov 2000). In this study, gray rockfish were found within the depth range of 83-390 m with mean depth of 186 m (there were no hauls shallower than 80 m). Maximum catches were made from less than 100 m to about 200 m (Fig. 2a). This probably reflects seasonal changes of depths inhabited. The gray rockfish during periods of mating (autumn) and larval release (spring-summer) occupies shallower depth in comparison with the rest of the year (Kondratiev 1996, Nemchinov 2001). Our observations (Fig. 3c) do not conflict with these data. However, the pattern of vertical distribution presented might be incomplete due to lack of trawling in shallow waters where the bulk of the population may exist.

The angry rockfish is the most deepwater species among rockfishes considered. It inhabits a depth range of 200 to 1400 m (Sheiko and Fedorov 2000), with optimal depths believed to be either 450-1,000 m (Fedorov 2000) or 400-800 m (Amaoka et al. 1995). In the study area, angry rockfish occurred in catches at depths of 370-650 m (mean 474 m). The maximum catch rates were registered within the depth range of 450-550 m and the diagram of vertical distribution had one peak only (Fig. 2b) which may be associated with the fact that this species is caught in the study area predominantly in the summer period. It is known that during the summer the angry rockfish migrates from feeding areas in deeper waters to shallower depths. In addition, it is known that this species inhabits shallower depths in the northern parts of the range as compared to the southern ones (Nagasawa and Torisawa 1991).

The northern rockfish within its range occupies depths of 73 to 740 m (Sheiko and Fedorov 2000) with an optimum between 95 and 190 m (Fedorov 2000), although optimal depths vary with location. According to data of Snytko (2001), maximum abundance of the northern rockfish occurred at 100-120 m in the western Bering Sea, at 100-400 m in its southeastern part, at 235-250 m off the Bowers Ridge, at 100-320 m off the Aleutians, and at 70-250 m in the Gulf of Alaska. According to other data (Clausen and Heifetz 2002), the northern rockfish is most abundant off the Aleutians at depths of 75-175 m, and in the Gulf of Alaska at 75-150 m. Off the Kurils and Kamchatka the maximum abundance of this species was registered at depths of 150-200 and 300-350 m (Fig. 2c). The existence of two peaks of vertical distribution is probably related to seasonal changes of depths inhabited since northern rockfish feed during summer months in shallower waters in comparison with the rest of the year (Love et al. 2002). Our observations (Fig. 3c) also showed that, overall, northern rockfish were distributed in slightly shallower waters in the summer months.

The relationship between capture depth and average body weight showed that gray rockfish tended to be larger in shallower waters, because juvenile fish lived in deeper waters (Fig. 3a). Such size-depth differentiation may serve to decrease intraspecific feeding competition.
Figure 3. Relation between average body weight and capture depth of (a) gray and (b) northern rockfishes, and monthly changes of their mean capture depth in Pacific waters off the northern Kuril Islands and southeastern Kamchatka, 1992-2002 (dashed lines indicate linear trend, $R = \text{correlation coefficient}$).
The smallest northern rockfish was registered at depths less than 250 m and over 300 m (Fig. 3b) while the largest fish was caught within 250-300 m depth range (differences are statistically invalid). However, similar size-depth distribution of this species were observed in the Gulf of Alaska and off the Aleutians (Harrison 1993, Ronholt et al. 1994, Martin 1997).

**Bottom temperature preference**

The gray rockfish in the Sea of Japan occupies a very wide range of bottom temperatures, from 1.5 to 15°C (Novikov et al. 2002). In the study area, this species dwelled in the coldest water among the three rockfishes considered (Table 1). It was registered in catches at bottom temperatures from –1 to 3.5°C (mean 1.4°C); about half of these fish were caught within the range of 0.6 to 1°C (Fig. 4a).

There are no data on temperature preferences of the angry rockfish. Our insufficient observations showed that this species occurred in relatively warm waters (Table 1). In the catches, it occurred within a very narrow range of bottom temperatures of 3.1 to 3.55°C (mean 3.41°C).
Figure 5. (a) Multi-annual, (b) seasonal, and (c) diurnal changes of catch rates and occurrence of gray rockfish in Pacific waters off the northern Kuril Islands and southeastern Kamchatka, 1992-2002 (bars are average catch, dashed line is occurrence).
Figure 6. (a) Multi-annual, (b) seasonal, and (c) diurnal changes of catch rates and occurrence of northern rockfish in the Pacific waters off the northern Kuril Islands and southeastern Kamchatka, 1992-2002 (bars are average catch, dashed line is occurrence).
The preference by angry rockfish of bottom temperatures over 3ºC confirms earlier suggestions (Orlov et al. 1998) that this species penetrates this area from the south during seasonal or long-term warming only.

The temperature ranges at which the northern rockfish occurred off the bottom differ in various areas. According to data of Snytko (2001), they are 3.1-4ºC in the southeastern Bering Sea, 2.1-4.4ºC off the western Aleutians, 2.9-5.8ºC off the eastern Aleutians, and 4.1-5.3ºC in the western Gulf of Alaska. By comparison, in the Pacific waters off the northern Kurils and southeastern Kamchatka, this species occurred at considerably lower bottom temperatures that varied from −0.2 to 3.6ºC (mean 1.9ºC). At the same time, more than half the specimens were caught within the temperature range of 2.1-2.5ºC (Fig. 4b).

**Multi-annual changes of occurrence and catch rate**

The occurrence and catch rate of species varied during the year. For most of the study period, catches of gray rockfish were rather low (Fig. 5a). Its occurrence slightly increased in 1999 and reached a maximum in 2001. All the captures of angry rockfish occurred in 1998, 1999, and 2000 though there were only two records of this species in 2000. There were no captures observed of the angry rockfish during other years. The captures of the northern rockfish in the area surveyed prior to 1996 were incidental (Fig. 6a). After 1997 its catches were very low but rates increased dramatically in 2001. It should be noted that catch dynamics of gray and northern rockfishes are very similar. That may be related to long-term changes of thermal conditions in the study area and also to similar temperature preferences of both species. The captures of angry rockfish in the study area were registered in 1998-2000, when other rockfish species occurred only occasionally. In 2001 catch rates of gray and northern rockfishes increased while angry rockfish disappeared from the catches.

**Seasonal changes of occurrence and catch rate**

The catch rate and occurrence of rockfish species exhibit seasonal dynamics that may be associated with different phases of their life cycles. The maximum catches of gray rockfish in the study area were registered in June and September while its maximum occurrence was in May-June and October-December (Fig. 5b). The density of gray rockfish concentrations increases during the mating and larvae release periods, which in the southwestern Sea of Okhotsk occurred respectively in September-October and May-June (Panchenko 1996, Nemchinov 2001). In the northern Sea of Okhotsk gray rockfish releases its larvae from late June to early July (Kondratiev 1996) but according to other data (Chereshnev et al. 2001), it happens from the end of summer to the beginning of autumn. Thus, maximum catches of gray rockfish in the area surveyed in June and September are most probably associated with
mating and larvae release which occurred there in the same periods as in other regions. The existence of gray rockfish spawning may be evidence of a separate population in Pacific waters off the northern Kurils and southeastern Kamchatka.

The limited data on angry rockfish captures off the Kurils showed that this species occurred here predominantly during summer months (July-August), i.e. when near-bottom layers have maximum water temperatures. Its captures in November and December 2000 off the southern tip of the underwater plateau were probably related to a delay of migration of some fish, whereas the majority of gray rockfish had already moved back to the south.

Occurrence of northern rockfish during the year varied insignificantly though its maximum catches were registered in May and August-September (Fig. 6b). In the Gulf of Alaska maximum catches of this species occurred in July and October; off the Aleutians they are observed from March to May (Clausen and Heifetz 2002). According to published data the release of larvae in the Northeast Pacific occurs in spring (Love et al. 2002); in the Bering Sea it happens in April-May (Moiseev and Paraketsov 1961). The first catch peak is probably associated with spawning concentrations. There are no data on the period when the northern rockfish is mating. Judging from the catch rates, the mating of this species in the area surveyed most probably occurs in August-September when its catches are maximal. In the Gulf of Alaska mating probably occurs in October when northern rockfish form the densest aggregations.

**Diurnal changes of occurrence and catch rate**

The diurnal dynamics of catch rate and occurrence of these rockfishes probably reflect the changes of their distribution patterns during the day, which may be related to changes of fish behavior and physiological conditions and, as a consequence, the daily variability of fish availability for fishing gear. The maximum gray rockfish catches occurred from midnight to 9 am (Fig. 5c). Large catches during that period may reflect peculiarities of feeding behavior. Within the entire species range gray rockfish diet consists mostly of gelatinous plankton (jellyfish) and euphausiids (Kondratiev 1996, Chereshnev et al. 2001, Novikov et al. 2002). In the study area, the gray rockfish also ate mainly jellyfish and fishes. It is possible that the density of gray rockfish prey within the near-bottom layer is maximal from midnight until 9 am, and highest catch rates during this time period are related to increasing feeding activity of these fish.

The angry rockfish was registered in catches most frequently (59.4%) between 9 am and 6 pm. In Japanese waters this species feeds on crustaceans, squid, and fish (Nagasawa and Torisawa 1991). In the area surveyed it ate mysids and shrimps. It is known that the angry rockfish
during night hours goes up in the water column to feed (Nagasawa and Torisawa 1991). This is the probable reason for its higher catch rates during the light period in comparison with dark.

The maximum catches of northern rockfish were also observed during daylight from 4 am to 6 pm (Fig. 6c). The bulk of its diet within the entire range consists of copepods and euphausiids (Skalkin 1964; Mito 1974; Brodeur and Livingston 1988; Yang 1993, 1996), i.e., of crustaceans performing daily vertical migrations. In the study area, the stomachs of northern rockfish mostly contained copepods and euphausiids. Apart from these dietary components it consumed arrow worms (Chaetognatha), comb jellies (Ctenophora), nudibranchs (Nudibranchia), midwater squid *Galyteuthis phyllura*, and bigeye lanternfish *Protomyctophum thompsoni*. It seems that during dark hours the northern rockfish migrates for feeding from the bottom into the water column together with plankton, and its catches in this period decrease.

**Length**

The data on maximum length of the gray rockfish are controversial. Some authors believe it is 50 cm (Amaoka et al. 1995, Orr et al. 2000, Love et al. 2002, Novikov et al. 2002) while others suggest that this species attains 56 cm (Snytko 2001) and even 59 cm (Chetvergov 1998, Chereshnev et al. 2001, Mecklenburg et al. 2002). The size composition of species considered depends on fishing gear and differs in various areas. Off the southern Kurils, gray rockfish taken by gillnets varied in length between 33 and 50 cm with a mean length of 40.5 cm (Panchenko 1996). Fish caught off southeastern Kamchatka by longline were considerably larger; their maximum and mean lengths were 59 cm and 52.8 cm respectively. At the same time, longline catches taken off the Commander Islands contained significantly smaller gray rockfish (range 27-46 cm, with mean length 36.2-40.1 cm). Even smaller fish were found in bottom trawl catches taken off western Kamchatka where length varied between 11 and 51 cm with mean value of 31.3 cm (Chetvergov 1998). The smallest gray rockfish probably inhabits the northern Sea of Okhotsk where catches are represented by fish ranging in length between 8 and 38 cm, and predominately 20-28 cm (Kondratiev 1996, Chereshnev et al. 2001). In the study area, bottom trawl catches comprised gray rockfish individuals measuring 19-57 cm with mean length 44.78 cm (Fig. 7a). Fish sized 38-42 and 49-53 cm were most numerous. In bottom trawl catches off the western Kamchatka, gray rockfish of 23-29 and 33-39 cm predominated (Chetvergov 1998). Thus, size structures of species considered in the area surveyed and off western Kamchatka differ considerably. This confirms our earlier conclusions that the gray rockfish is a permanent dweller of Pacific waters off the northern Kurils and southeastern Kamchatka and doesn’t migrate there from
Figure 7. Length frequencies of (a) gray, (b) angry and (c) northern rockfishes in the Pacific waters off the northern Kuril Islands and southeastern Kamchatka, 1992-2002 (n = number of fish measured, M = mean length).
the Sea of Okhotsk. In longline catches taken adjacent to our areas off southeastern Kamchatka and the Commander Islands, gray rockfish of 47-57 cm and 37-43 cm size classes, respectively, were most abundant (Chetvergov 1998). This indicates significant similarity of size composition of fish in all three above-mentioned areas.

Previously, angry rockfish were noted to attain 60 cm in length (Masuda et al. 1984, Nagasawa and Torisawa 1991). Our catches were represented by fish of lengths between 26 and 67 cm with mean value 46.55 cm, and most individuals sized 40-44 cm (Fig. 7b).

Maximum size of the northern rockfish has been variously reported as 40 cm (Orr et al. 2000), 41 cm (Kramer and O’Connell 1995, Mecklenburg et al. 2002), 42 cm (Snytko 2001), and 48 cm (Clausen and Heifetz, Love et al. 2002). Size composition of this species differs considerably among different areas. On the whole, the fish from the Gulf of Alaska are notably larger than those off the Aleutians (Yang 1993, 1996; Clausen and Heifetz 2002). The smallest northern rockfish inhabits the southern Bering Sea (individuals sized 16-17, 20-23, and 26-27 cm prevalent, mean length 24.4 cm); off the Aleutians specimens with length 27-32 cm (mean 30.2 cm) are most numerous in catches; fish from the eastern Bering Sea (mean length 34.1 cm) are slightly larger; the largest northern rockfish are taken in the Gulf of Alaska where the mean length is 37.3 cm and most abundant are specimens having length 35-40 cm (Bakkala et al. 1992, Harrison 1993, Martin 1997). Catches in the study area were represented by fish with length ranging from 15 to 48 cm with a mean value of 32.4 cm. Individuals of two size groups, 27-31 and 34-38, cm (Fig. 7c) were most numerous, i.e., similar to the length classes in the Gulf of Alaska and off the Aleutians.

**Body weight**

There are few data on maximum body weight of these species. It is believed gray rockfish may attain 1.9 kg (Chereshnev et al. 2001). In longline catches off southeastern Kamchatka gray rockfish body weight ranged from 0.6 to 2.95 kg (mean 2.15 kg) with most fish weighing 1.25-1.75 kg (Chetvergov 1998). The gray rockfish taken by bottom trawls in the northern Sea of Okhotsk and off western Kamchatka were significantly lighter with body weights of 5.2-874 g (Kondratiev 1996) and 20-950 g (Chetvergov 1998). Near the Commander Islands longline catches were represented by fish with body weights of 0.4-1.8 kg with mean values of 0.9-1.2 kg (Chetvergov 1998). In the study area, the gray rockfish body weight was 0.140-3.3 kg with mean 1.8 kg (Table 1). Taking into account differences between fish sizes of trawl and longline catches, it is possible to conclude that our data on body weight from the area under study is very close to those from southeastern Kamchatka waters (Chetvergov 1998).
Figure 8. Length-weight relationships of (a) gray, (b) angry, and (c) northern rockfishes in the Pacific waters off the northern Kuril Islands and southeastern Kamchatka, 1992-2002 (n = number of fish measured).
Published data on body weight of the angry rockfish is lacking. In the study area its individuals had body weights of 0.47-3.7 kg with mean 1.46 kg (Table 1).

It is known that the northern rockfish may attain weights of 1.8 kg (Snytko 2001). Gulf of Alaska fish are distinguished by maximum body weight; the mean values varied from 0.68 to 0.95 kg for different years (Martin 1997, Clausen and Heifetz 2002). Individuals taken in the eastern Bering Sea are characterized by a somewhat smaller body weight (mean 0.676 kg) but smallest northern rockfish (mean value range 0.35-0.59 kg in different years) are caught off the Aleutian Islands (Bakkala et al. 1992, Ronholt et al. 1994, Clausen and Heifetz 2002). In the area surveyed the species in question had body weights range 80-1200 g with a mean of 602.7 g (Table 1), and according to this pattern fish taken here and off the Aleutians were quite similar.

**Length-weight relationship**

There are a few published data regarding length-weight relationships of the three rockfish species. This relationship allows judging the character of fish growth (Zotina and Zotin 1967) while the power coefficient of its equation may vary among fish belonging to different populations (Vinberg 1971). There are no published data on length-weight relationships for the gray rockfish. In the study area length and weight of species considered had a well-pronounced relation \((R^2 = 0.881)\). The power coefficient of this equation proved to be close to three (Fig. 8a), which is characteristic of the majority of aquatic animals (Vinberg 1971).

There are no data on length-weight relationships of the angry rockfish as well. Off the Kurils this relationship had a high correlation \((R^2 = 0.954)\) and the power coefficient was notably larger than three (Fig. 8b), which may be evidence of different growth patterns of both species described.

There are some data on length-weight relations of the northern rockfish from off the Aleutians (Ronholt et al. 1994) and the Gulf of Alaska (Martin 1997). In the Aleutians the linear coefficients depending on the study year ranged from 0.0151 to 0.0314 while the power ones varied from 2.78 to 2.996. In the Gulf of Alaska respective values were 0.0124 and 3.035. In Pacific waters off the northern Kurils and southeastern Kamchatka the relationship considered was quite well expressed \((R^2 = 0.877)\). Linear and power coefficient of respective equations (Fig. 8c) were very similar to those from the Aleutian Islands waters.

**Sex ratio, sexual dimorphism in sizes, stomach fullness**

It is known that one male gray rockfish will mate with several females (Chereshnev et al. 2001). Such a spawning feature suggests numerical predomination of females in populations. However, in the majority of areas male gray rockfish are more abundant than females. Off the
southern Kurils the sex ratio was 68% vs. 32% (Panchenko 1996); in the northern Sea of Okhotsk it was 65% vs. 35% (Kondratiev 1996); off the southeastern Kamchatka it was 69.5% vs. 30.5% (Chetvergov 1998). Near the western Kamchatka coast the sex ratio of the gray rockfish is almost equal while off the Commander Islands females are more abundant and prevailed over males 59% vs. 41% (Chetvergov 1998). In the study area, similar to many other parts of its range, female gray rockfish were also less abundant than males (33.9% vs. 66.1%).

It is also known that male gray rockfish in most areas are larger than females (Kondratiev 1996, Nemchinov 2001). Thus off western Kamchatka mean lengths and weights of males were 40.1 cm and 1.2 kg, and mean lengths and weights of females were 36.2 cm and 0.9 kg. In the area surveyed males were also larger than females. Mean values of body length and weight were 48.82 and 41.65 cm and 2,286.6 and 1,415.6 g respectively.

Feeding intensity of the gray rockfish during the year is rather low; average stomach fullness index off the southern Kurils were 0.9 in June, 1.4 in July, and 1.2 in October (Nemchinov 2001). In the study area stomach fullness of the species considered was even lower (SFI 0.16 on average). Since the maximum catches were taken in September (mating period), low feeding activity is probably related to almost no feeding in this time. It should be noted that female stomach fullness (SFI 0.47 on average) was slightly higher than in males (SFI 0.03 on average).

Sex ratio and sexual dimorphism of the angry rockfish are unstudied. In Pacific waters off the northern Kurils the number of males and females in catches was close to equal (51.6 vs. 48.6% respectively). Male angry rockfish were notably larger than females. Their mean lengths and weights were 45.88 and 42.93 cm and 1,663.1 and 1,248.7 g respectively. The feeding intensity of the angry rockfish during summer months was quite low. The average stomach fullness index was 0.67; during the same time females consumed larger amounts of food (average SFI 0.93) in comparison with males (average SFI 0.33).

According to published data (Westrheim and Tsuyuki 1971, Clausen and Heifetz 2002), in the Gulf of Alaska the sex ratio of northern rockfish in catches is almost equal with minor female prevalence (50.8 vs. 49.2%). Off the Aleutians predominance of females is considerably greater, 57.1 vs. 42.9% (Clausen and Heifetz 2002). The northern rockfish sex ratio in the area surveyed was quite similar to that described above (59.3% females and 40.7% males).

Some authors (Westrheim and Tsuyuki 1971) suggest a similar male and female size composition in the Gulf of Alaska, while others (Clausen and Heifetz 2002) noted greater female length compared to males: 34.3 and 33.0 cm in the Gulf of Alaska and 30.8 and 29.1 cm off the Aleutians. In the study area males were slightly longer than females (34.1 and 33.9 cm) though females were notably heavier than males (672.5 and
617.3 g). The northern rockfish fed little during the study period; the average value of SFI was 1.0. During the same time, females consumed food somewhat more intensively (average SFI 1.33 in females vs. 0.55 in males).

References


Barsukov, V.V. 1981. Brief review of the rockfish subfamily system (Sebastinae). Vopr. Ikhtiologii 1:3-27. (In Russian.)


Zotina, R.S., and A.I. Zotin. 1967. Quantitative relationships between weight, length, age, egg size, and fecundity in animals. Zhurnal Obshchей Biologii 1:82-92. (In Russian.)
Preliminary Results of Trans-generational Marking of Larval Marine Fish Otoliths

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Abstract
Dispersal, connectivity, and retention of larval fish are key ecological processes affecting populations of marine fishes. Quantification of these parameters is vital for effective use of marine reserves and other resource management options, and yet these determinations are among the greatest challenges facing marine ecologists today. A major impediment is the lack of a reliable technique for marking extremely small marine fish larvae. Extensive testing with captive rockfishes (Sebastes spp.) and surfperches (Embiotocidae) has validated that trans-generational mass marking of larvae in vivo occurs with the transfer of elemental strontium to otoliths of developing larvae via matrotrophic viviparity. The mark is induced by intramuscular injection of up to 30,000 ppm strontium chloride into gestating females in situ. The marks are permanent, and can provide unique identifiers for cohort and location. Laser ablation inductively coupled plasma mass spectrometry detects marks in juvenile otoliths as a zone of significantly increased ratio of strontium to calcium. The first field test of trans-generational marking used brown rockfish (S. auriculatus) on Pt. Heyer reef in Puget Sound, Washington. Post-settlement juveniles were captured for otolith recovery only from Pt. Heyer reef. To date, 127 otoliths from marked-cohort juveniles have been analyzed and one strontium-marked otolith recovered.
Introduction

Understanding the patterns of larval marine fish dispersal that result in either connectivity between distant populations, or retention in local populations, or both, is key to understanding a host of ecological factors that influence populations of marine fishes. The consequences of incorrect predictions about distant larval dispersal and population connectivity, versus local retention, can have profound implications for global, regional, and local approaches to marine fish management (Roberts 1997, Palumbi 1999, Cowen et al. 2000, Lockwood et al. 2002, Warner and Cowen 2002, Botsford et al. 2003). This information is also vital for effective resource management interventions in these natural processes, such as marine reserves, that are designed to sustain, rebuild, or enhance the populations. Some current management approaches emphasize the use of marine reserves to protect habitat, biodiversity, and spawning biomass in order to enhance progeny output that will contribute to local and/or regional stocks. Use of marine reserves assumes that natural patterns of larval dispersal will accomplish these goals. However, the dispersal of larval fish is among the least understood aspects of marine fish ecology, and the extent of larval movement away from source populations is rarely known.

The assumption, that populations of marine fish operate as “open” systems and larval recruitment into a population is independent of local production, is being reconsidered in light of increasing evidence of larval fish retention near source populations (Jones et al. 1999; Swearer et al. 1999, 2002; Jones et al. 2005). Retention of larvae is also supported by evidence that many marine fish larvae have the ability to detect and respond to environmental cues by altering their dispersal patterns, and these larvae should not be considered as passively dispersed particles (Kingsford et al. 2002). If the recruitment of marine fish larvae and pelagic juveniles represents local retention, then the design of effective marine reserves must include preservation of retention areas as well as adult habitats (Warner and Cowen 2002).

Recent applications of molecular genetic techniques (Strathmann et al. 2002, Swearer et al. 2002, Buonaccorsi et al. 2004, Hauser et al. 2007), and mass spectrometric analysis of otolith microstructure and microchemistry (Jones et al. 1999, Swearer et al. 1999, Guido et al. 2004, Miller and Shanks 2004, Warner et al. 2005), have improved knowledge of larval fish dispersal patterns. However, these techniques yield estimates of larval dispersal or retention on broad geographical and population-level scales and seldom provide information on exact larval-source locations (Hellberg et al. 2002). Definitive information is needed on larval fish dispersal that is based on direct measurements that connect the exact origins of the larvae with the subsequent recruitment of juveniles to specific locations. Such measurements are key to determining the connectivity of populations of marine fishes, and the
effects of larval retention and dispersal processes on the variability of this connectivity.

Quantification of dispersal and retention rates is a major hurdle in marine ecological studies that has been described as “one of the greatest challenges facing marine ecologists today” (Swearer et al. 2002). The difficulties in achieving this goal for marine fishes are compounded by extremely small pelagic larvae that suffer high mortality rates and that may disperse over large areas for months. Current analytical techniques are inadequate to mark the large number of larvae needed to achieve mark recoveries at later life stages (Swearer et al. 2002, Thorrold et al. 2002, Miller and Shanks 2004). The only unambiguous way to quantify dispersal and retention rates is through the recovery of artificial marks or tags that can be used to track larvae from specific origins to ultimate destinations (Jones et al. 1999, Thorrold et al. 2002).

Torrrold et al. (2002) note that the difficulties in marking large numbers of larvae may be alleviated by having “a tag that could be transferred from the female to developing eggs or embryos.” In this study, we describe and test a trans-generational marking technique that uses natural processes in viviparous fishes (Sebastes spp., Embiotocidae) to transfer an artificial body burden of naturally occurring elemental strontium (Sr) from gestating females to their larvae. The in vivo exposure of the larvae to Sr levels well in excess of ambient induces a permanent Sr mark in the otoliths of the larvum prior to parturition.

Methods

Trans-generational marking

In this study, trans-generational marking uses intramuscular injections of elemental Sr, in a strontium chloride (SrCl₂) solution, in late-stage gestating viviparous female rockfishes (Sebastes spp.) and surfperches (Embiotocidae) to elevate the Sr level in the ovarian fluid. Strontium is a naturally occurring element in seawater that is found in all bony structures in marine fishes. The conduit for transferring the Sr to the larvae in vivo is the natural matrotrophic viviparity in these fishes, whereby the females supply nutrition to the developing embryos (Turner 1938, 1952; Dygert and Gunderson 1991; Shimizu et al. 1991). Injection dosages were 1 ml per 500 g body weight of 30,000 ppm SrCl₂ in an isotonic solution (0.1125 M SrCl₂·6H₂O). Injections were intramuscular; half of the dose was injected into each side of the fish, near the proximal margin of the spiny dorsal between the 5th and 7th rays. Extensive testing with captive brown rockfish (S. auriculatus), kelp perch (Brachyistius frenatus), and shiner perch (Cymatogaster aggregata) confirmed that trans-generational mass marking of the larvae in vivo results in otoliths with permanent marks (R. Buckley and E. Volk, Washington Department of Fish and Wildlife (WDFW), unpubl. data).
Field trials of trans-generational marking could not proceed until the U.S. Food and Drug Administration (FDA) determined that injections of elemental Sr in adult rockfish, at dosages significantly higher than ambient, would not pose a threat to human health if injected rockfish were consumed post-injection. Pivotal research on Sr depuration rates in kelp rockfish (*S. atrovirens*) answered FDA concerns (D. Casper, M. Carr, P. Raimondi, and N. Grant, University of California Santa Cruz, unpubl. report to FDA), and in spring 2004 the FDA granted regulatory discretionary authority to inject and release brown rockfish, kelp perch, and shiner perch. Field trials were designed to evaluate both the utility of applying trans-generational marking procedures in situ under field conditions, and the recovery of Sr-marked otoliths from post-settlement juveniles.

**Rockfish field trial**

The field trial was conducted at Pt. Heyer reef, located in central Puget Sound, Washington, on the eastern shoreline of Vashon Island (47°25.2'N, 122°25.6'W). This insular reef was constructed of high-relief quarry rock and large concrete material in 1983 (2,500 m²; Hueckel and Buckley 1987), and modified in 1991 with the addition of small rock (8-10 cm diameter; 3,000 m²; West et al. 1995) in a low-profile design to create nursery habitat for juvenile rockfishes. The total reef coverage of 5,500 m² encompasses a bottom area of 7,000 m², and depths of –4 m to –36 m mean-lower-low-water (MLLW) on a moderately sloping sand and gravel substrate. Pt. Heyer reef is separated from other rock-reef habitat by >7 km in all directions.

Pt. Heyer reef typically had an adequate number of reproductive-sized adults (approximately 200-250 fish >20 cm total length (TL); WDFW, unpubl. data) for experimental purposes. Females with an enlarged abdomen, indicating late-stage gestation, were captured during spring-summer 2004 on the reef by divers using 30 cm diameter hand nets with 2 mm red mesh, and transferred to a holding bin placed on the bottom at capture depth. The holding bin was moved, if necessary, to a depth of 10 m for injection operations to optimize diver bottom-time constraints.

Injections of SrCl₂ were applied in situ within 1-2 hours post-capture in order to avoid subjecting the fish to barotraumas and to minimize handling stress. Fish were measured to the nearest cm TL to estimate weight from a length-weight table, and the upper lobe of the caudal fin was clipped to obtain a tissue sample for genetic analyses in a companion study (see Hauser et al., 2007). The caudal fin-clip also temporarily marked the injected females to avoid recapture. Injections were administered using a 1 ml dosage-adjustable, self-refilling syringe fitted with a 21-gauge hypodermic needle. The syringe was coupled with surgical
tubing to a 1.8 L hydration reservoir (Platypus™) filled with SrCl₂ solution. Injected fish were immediately released onto reef habitat.

Juvenile brown rockfish (i.e., young-of-the-year) were captured on Pt. Heyer reef using 15-30 cm diameter hand nets with 2 mm red mesh. Tissue samples for genetic analyses (see Hauser et al. 2007) were taken from the juveniles in the field. Otoliths (sagittae) were recovered in the laboratory, cast in resin blocks, and prepared for mark recovery analysis as described in Volk et al. (2000). Mark recovery analyses were conducted using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS; see Miller and Shanks 2004), and marked otoliths were identified by a zone that had a substantially increased ratio of strontium to calcium (Sr:Ca) near the core of the otolith.

**Fecundity analysis**

Gestating brown rockfish (n = 6, range 21-37 cm TL) captured in June 2004 on central Puget Sound reefs provided ovary samples with developing larvae from which estimates of fecundity were made by extrapolating weights of enumerated subsamples of larvae from each ovary to the total weight of the ovary. A length-fecundity relationship was estimated using linear regression (Neter et al. 1990).

**Results**

In 2004, injections of SrCl₂ were administered to 31 gestating brown rockfish that ranged in size from 21 to 31 cm TL and averaged 25 cm TL (Table 1). The six gravid brown rockfish used for fecundity estimates encompassed the size range of the 31 injected rockfish, and provided a preliminary length-fecundity relationship for estimating the number of larvae in the rockfish injected with SrCl₂ (Adj. $R^2 = 0.93$, $F_{(1,4)} = 71.8$, $p = 0.001$). The estimated numbers of developing larvae in the injected rockfish ranged from 3,506 in the 21 cm TL female to 138,304 in the 31 cm TL female. Approximately 1.7 million larvae were exposed to Sr in vivo (Table 1), assuming 100% efficacy of the treatment.

In September and October following the summer parturition period for brown rockfish, fish that were ≤100 mm appeared in nearshore reef habitat (i.e., depths of 8-18 m). It was assumed that fish of this size range on Pt. Heyer reef had recently recruited to the reef (Fig. 1). This size-group also exhibited the “typical” new recruit behavior of being closely associated with, and often hidden in, small-sized crevice habitats in the rock substrate (see Buckley 1997). We collected 2004 cohort Pt. Heyer juveniles (n = 325, range 45-100 mm TL) for otolith samples from October 2004 through June 2005.

Limitations in the availability of the LA-ICPMS equipment resulted in Sr analysis of only 127 of the 325 otoliths. Those analyses determined that 126 of the otoliths had Sr:Ca ratios across the otolith that was
Table 1. Data for 31 gravid brown rockfish injected with 30,000 ppm SrCl\(_2\) at Pt. Heyer reef in 2004, to induce in vivo trans-generational Sr marks in the otoliths of developing larvae. See text for length-fecundity estimation.

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<th>Capture depth (m)</th>
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consistent with exposure to ambient concentrations of these elements. Those otoliths were classified as non-Sr-marked otoliths (Fig. 2A). One otolith also had strong spikes in the Sr:Ca ratio on either side of the core region, which demonstrated that the larva had been exposed to a >200% increase in Sr for a short period during early development (Fig. 2B). This otolith was classified as having a definite Sr mark. The two spikes in the Sr:Ca ratio represented the laser passing through a circular band (i.e., a growth increment) around the otolith core that contained an elevated concentration of Sr.

The Sr-marked juvenile brown rockfish was 60 mm TL when it was collected on January 27, 2005. A companion genetic study to determine parent-progeny relationships of brown rockfish on Pt. Heyer reef determined that the Sr-marked juvenile was the progeny of a 22 cm TL female captured on Pt. Heyer reef on July 9, 2004, and injected with SrCl₂ solution as part of this study (see Hauser et al. 2007). The juvenile was collected 202 days post-injection. During the injection process, that female released a small number of premature but well developed larvae, indicating that parturition would likely occur soon and that the developing larvae would be exposed to high Sr levels in vivo for only a short period of time.

Figure 1. Length frequency distribution, by month, of 2004 cohort juvenile brown rockfish ≤100 mm total length (n = 359), collected on Pt. Heyer reef, May 2004 through June 2005. (Note: Collection months are numbered as January = 1 to December = 12, and juvenile rockfish are grouped by month regardless of collection year.)
Figure 2. LA-ICPMS analysis of 2004 cohort juvenile brown rockfish otoliths from Pt. Heyer reef. A: Non-Sr-marked otolith; B: Sr-marked otolith. (Note: The laser moves across the otolith (from left to right) recording Sr:Ca ratios in the matrix of the otolith. A: Sr:Ca ratios show exposure to ambient concentrations of these elements; B: Sr:Ca ratios show marks from exposure to high Sr concentrations for a short period early in larval development, and exposure to ambient concentrations of these elements over the remainder of the otolith.)
Discussion

Rockfishes and surfperches are ecologically important species in temperate marine reserves, and it is critical to quantify the dispersal and retention rates of their larvae to determine the efficacy of current marine reserve designs and locations. Trans-generational mass marking provides the first method for using a specific manipulation of gestating viviparous fishes to induce a specific mark in the otoliths of the larvae in vivo. The larvae are born carrying a benign permanent artificial "tag" that can be used to identify different cohorts and marking locations over the life of the fish.

The field trial with brown rockfish demonstrated the utility of this method when applied in situ, and that it is likely applicable to all rockfishes. The recovery of one Sr-marked otolith from 127 juveniles further demonstrated that trans-generational marks in larvae can be recovered from post-settlement juveniles. These validations are reinforced when the relatively small sizes of the experimental groups are considered. Only 31 brown rockfish females were injected, and approximately 1.7 million larvae were exposed to Sr in vivo assuming 100% efficacy. The latter is not an enormous number considering the potentially high natural mortality rates of rockfish larvae, and that post-settlement juveniles were the earliest development stage examined for Sr marked otoliths. The underwater injection protocols likely enhanced the potential for success by minimizing stress to the females, and reducing possible mortality or alteration of behavior due to handling. It was common to see females on the reef that had been injected with SrCl₂ many days earlier (identified by the genetic sample fin-clip in the upper lobe of the caudal) demonstrating the same behaviors as non-injected fish.

The field trial did not prove that all the injected females proceeded to term and released viable larvae. Validating these parameters would require tracking injected females and observing parturition of live larvae under natural conditions. This would be an extremely difficult task. The next best, albeit indirect, proof that trans-generational marking with SrCl₂ does not affect the development or survival of viviparous fish larvae comes from studies done with rockfishes and surfperches held in captivity to develop the marking technique. Captive brown rockfish commonly progressed through parturition and released live larvae after being injected with SrCl₂ at 30,000 ppm, and larvae could be reared for several weeks. However, the small size of the larvae (5-6 mm TL) precluded definitive analyses of the otoliths to verify exposure to Sr during development, as well as accurate assessments of meristics for comparisons of larval development in control and experimental groups. Better information can be gained from kelp perch, which release “early-stage” juveniles (35-45 mm TL) at parturition that readily feed in captivity and can be reared. Juveniles from female kelp perch injected
with SrCl$_2$ at 30,000 ppm during gestation had strong Sr marks in their otoliths (Buckley et al., WDFW, unpublished data). Also, comparisons of meristics of these juveniles from control and experimental groups detected no significant differences in paired structures within groups, or in single and paired structures between groups. These comparisons are commonly used indicators of abnormal development.

In the field trial it was assumed that all of the larvae developing in the ovary of a female injected with SrCl$_2$ are adequately exposed to the high Sr levels, both in duration and at concentrations to induce a detectable mark in the larval otolith. The physiological work on ovarian fluid dynamics and related larval development needed to validate this assumption remains to be done. However, Sr-marking the otoliths of early development stages of other species has demonstrated that a brief exposure to a relatively low concentration of Sr is sufficient to induce a detectable mark in the otolith (Schroder et al. 1995).

Given these potential sources for reduction in the number of Sr-marked juvenile brown rockfish available for recovery, it is an even more remarkable and conservative result that one mark was found in 127 otoliths. These potential reductions were offset somewhat (although to an unknown degree) by the potential for local larval retention and self-recruitment at Pt. Heyer reef. The configuration of the shoreline, tidal current gyres, and prevailing wind directions in the area may have created good conditions for retention of pelagic larvae. The potential for recruitment of brown rockfish larvae back to Pt. Heyer habitat is also enhanced if the late-stage larvae and early stage pelagic juveniles have the ability to alter their dispersal patterns and actively seek rocky-reef settlement habitat (see Kingsford et al. 2002).

More juvenile brown rockfish otoliths from Pt. Heyer reef, and from other locations, need to be analyzed, and more Sr-marked otoliths recovered, before the rate of local retention of larvae at Pt. Heyer can be determined. However, this preliminary field trial represents the first known recovery of a trans-generationally marked fish from the wild. This technique shows great potential for providing direct assessments of local retention and dispersal in marine fish larvae. Quantification of these parameters is critical for the marine ecological studies needed to design effective systems of marine reserves.

References


Genetic Identification of Progeny of Reef-Resident Brown Rockfish (*Sebastes auriculatus*)

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**Abstract**

The extent of larval retention and natal homing in demersal fish is a topic central to the design and the efficacy of marine protected areas (MPAs). Unfortunately, little is known about effective larval dispersal in many marine species. The duration of the pelagic phase in many species suggests extensive dispersal, and population genetic studies indicate large-scale exchange of migrants, though there is also recent evidence for surprisingly limited realized dispersal. Here, we use genetic markers (microsatellites) to identify the offspring of resident adult brown rockfish (*Sebastes auriculatus*) among incoming settling juveniles on an isolated artificial reef at Point Heyer in Puget Sound, thus directly estimating rates of self-recruitment on the reef. Due to low marker variability, unambiguous identification of these offspring from empirical data was not possible. Nevertheless, comparison between parent-offspring matches in observed and simulated genetic data suggested that self-recruitment was less than 10%. One of the juveniles genetically matching an adult was confirmed as its offspring by larval otolith marking, which confirms that self-recruitment does occur. Our data suggested
some, but limited, self-recruitment, that corresponded well to expectations at this scale from mean dispersal distances in brown rockfish.

**Introduction**

The extent of dispersal in marine species has attracted great interest among ecologists, evolutionary biologists, and resource managers alike, not only because data on dispersal and retention mechanisms provide powerful insights into the distribution, phylogeography (Feral 2002), and evolution of marine species (Palumbi 1996, Lessios et al. 2001), but also because assumptions about self-recruitment of marine stocks underlie many of the commonly used strategies in fish stock assessment (Cowan and Shaw 2002) and conservation (Planes et al. 2000). With the emphasis on marine protected areas (MPAs) as a tool for marine conservation, the question of realized dispersal of pelagic larvae has found renewed significance, because the function of MPAs in a regional context depends critically on the demographic exchange between the MPA and surrounding areas (Botsford et al. 2003, Palumbi 2003). At one extreme, retention of all life history stages within an MPA negates any positive effects on surrounding areas, while at the other extreme, total export of larvae or juveniles from the MPA may limit the conservation value of the protected area (Palumbi 2003). Some information on realized dispersal from MPAs is therefore required; and, although data on adult migration are accumulating (Pittman and McAlpine 2003), little is known about the effect of larval dispersal, which most likely dominates the level of demographic connectivity of protected areas with surrounding regions.

Unfortunately, there is little information on realized dispersal of marine larvae, and most estimates have been derived indirectly by inferences from current speeds, larval duration, or the genetics of adult populations (Bohonak 1999, Palumbi 2003). Such estimates are inherently imprecise; and, although cross-species correlations between genetic population differentiation (estimating migratory exchange) and larval duration could be demonstrated (e.g., Doherty et al. 1995, Bohonak 1999), the predictive value of such indirect inferences for MPA design remains limited. Furthermore, while some more direct evidence, such as the occurrence of larvae of coastal species in the open ocean (Scheltema 1986) and the rapid spread of marine invasive species with pelagic larvae (e.g., green crab, *Carcinus maenas*, Geller 1994) clearly demonstrate the occurrence of long-distance dispersal, its frequency and thus ecological significance in the short term, especially for MPA design, remains questionable (Palumbi 2003).

Indeed, recent evidence from larval biology suggests that long distance dispersal, although important evolutionarily (Strathmann 1978, Duda and Palumbi 1999), may be rare (Palumbi 2001) and that at least
some of the recruitment in demersal fish and benthic invertebrates may stem from local sources. Oceanographic features such as currents or eddies play an important role in dispersal and retention of pelagic larvae and may also strongly affect larval mortality (Bailey et al. 1997, Withler et al. 2001). Furthermore, behavioral mechanisms, such as vertical migrations exploiting currents at different depths, can greatly influence the direction and extent of horizontal advection (Bilton et al. 2002). Evidence for localized self-recruitment comes from unexpected genetic subdivisions in marine species (Avise 1992, Taylor and Hellberg 2003), the persistence of demersal fish with pelagic larvae on isolated oceanic islands (Hourigan and Reese 1987, White 1998), and information on larval distribution (Bailey et al. 1997, Hay and McCarter 1997) and behavior (Bilton et al. 2002). However, although such evidence suggests predominantly localized recruitment, the quantification of larval export vs. self-recruitment, which is so important for MPA design, remains elusive.

Two major approaches to the estimation of larval dispersal are particularly noteworthy; both have striking results, but they also have some complications. First, chemical signatures within the otoliths, either natural or artificial, can be used to track down the origin of juvenile fish. For example, by marking eggs of coral reef damselfish, *Pomacentrus amboinensis*, with tetracycline, a compound producing fluorescent marks in larval otoliths, Jones et al. (1999) demonstrated that 15-60% of larvae originated from the local adult population on Lizard Island, Great Barrier Reef (about 20 km² area). Although that paper clearly demonstrated a relatively high degree of self-recruitment, the confidence limits of the quantitative estimate were wide, mainly due to the large size of the adult population, which limited the proportion of marked eggs to 0.5-2%. A similar approach that exploited natural differences in otolith microchemistry was used to estimate self-recruiting rates of 60-81% to natal spawning sites in weakfish (*Cynoscion regalis*), an estuarine spawning fish in the eastern United States (Thorrold et al. 2001). The estimates of self-recruitment were more precise for weakfish, though the technique relied on differences in chemical composition of the water in natal habitats and may have been limited to estuarine species or more large-scale investigations.

The second main approach used for the estimation of effective larval dispersal is based on genetic differentiation among populations. Most genetic studies find only slight, if any, genetic differentiation among populations of marine species, which greatly complicates the interpretation of data in an applied context, because populations that exchange very few migrants (<10 individuals per generation) cannot be distinguished from a single larger randomly interbreeding population. Under such circumstances, classical population genetic analyses based on Wright’s $F_{ST}$ statistic are inadequate to estimate dispersal on
ecological timeframes (Waples 1998). However, new approaches to data analysis that used more realistic models now allow the estimation of mean dispersal distances from low but significant genetic differentiation (Palumbi 2003). Available genetic data suggest mean dispersal distances of 25-150 km in many marine invertebrate and fish species, an estimate that is consistent with observations from invasive species (Shanks et al. 2003). Although this approach revolutionized our perception of large scale or even ocean-wide random interbreeding, it is limited to species with a detectable increase in genetic divergence with geographic distance. In many species, sharp genetic breaks can be detected (Avise 1992, Taylor and Hellberg 2003), but in others there is no clear geographic pattern of genetic divergence (Hauser and Ward 1998). Furthermore, dispersal distance estimates are derived as a mean over wide geographic areas; and, although currents can be incorporated into the models (Palumbi 2003), the prediction of dispersal patterns at specific MPAs is still difficult.

An alternative and potentially very powerful approach to evaluate larval dispersal and retention is the use of molecular markers for parental assignment, which allows identification of recruits originating from local adult fish. Until recently, such parental identification was not feasible because of low variability of markers, lethal sampling, and time-consuming analysis of samples. However, the development of microsatellites as high-variability molecular markers now allows parental identification in wild populations. Microsatellites consist of 1-5 base pair (bp) repeats that form tandem arrays up to 300 bp in length and exhibit high levels of allelic variation in repeat number. Polymorphism exhibited by specific microsatellites is readily detected by amplification of the microsatellite by the polymerase chain reaction (PCR) and estimation of length variation on automated systems. Microsatellites have recently come into widespread use in kinship analyses (reviewed in Wilson and Ferguson 2002) because they offer three critical advantages: opportunities for nonlethal sampling, rapid analysis of samples, and high variability. The approach is currently used extensively in salmonid populations (Bentzen et al. 2001) mainly to estimate reproductive success (Dickerson et al. 2002, Seamons et al. 2004), interactions between wild and hatchery fish (McLean et al. 2003), and the evolution of life history strategies (Garant et al. 2003). A similar approach is also possible for marine fishes, provided that a large proportion of resident adults can be sampled, which increases the chance of detecting at least some offspring with practical sample sizes. That way, offspring could be assigned not only to adults on a specific reef or MPA, but in contrast to otolith tagging studies, also to individual adult fish. By selecting a species with relatively isolated adult populations of small size, which allows the collection of a high proportion of the breeding adults in a specific area, more accurate estimates of self-recruitment could be derived.
Here, we used parental assignment of recruiting juveniles as an alternative approach to estimating the level of self-recruitment and larval dispersal in brown rockfish (*Sebastes auriculatus*). We chose brown rockfish as a target species for several reasons: (1) adult brown rockfish have relatively small home ranges, and rarely move farther than 3 km (Matthews 1990, Stout et al. 2001), which minimizes influx of new and unsampled parents; (2) relatively small and insular populations of brown rockfish inhabit several artificial reefs in Puget Sound, which allows the collection of a large proportion of potential parents; (3) some of these artificial reefs have integrated and adjacent nursery habitat for the collection of recruiting juveniles; and (4) the feasibility of inducing trans-generational chemical marks in otoliths of larvae has been demonstrated recently in brown rockfish (Buckley et al. 2007), which provided an independent verification of genetic assignments.

**Materials and methods**

**Sampling**

The study site was an artificial reef at Point Heyer, on the eastern shoreline of Vashon Island in Puget Sound (47°25.2'N, 122°25.6'W, Buckley et al. 2007). The reef is relatively isolated from other rockfish habitat, and the next artificial reef is more than 7 km away. Adjacent to the reef is smaller boulder habitat suitable for juveniles and allowing the collection of settling recruits. During summer 2004, a total of 137 adult brown rockfish (>20 cm) were caught with hand nets by scuba divers, fin clipped, and released immediately. Thirty-one females were also injected with strontium chloride (SrCl₂) solution (see Buckley et al. 2007). Between spring 2004 and spring 2005, 209 recruits (<101 mm) were collected from the adjacent nursery habitats by divers who used hand nets. Fin clips of adults and juveniles were stored in 95% ethanol until analysis.

**Molecular methods**

DNA was extracted from each sample using DNeasy extraction kits (Qiagen), following the manufacturer’s protocols. Thirty-five microsatellite loci from eight *Sebastes* species were PCR-amplified following the original protocols for each locus, though annealing temperature and number of cycles were adjusted as needed to optimize PCRs. Of the 35 loci screened, 13 loci from five species were selected for further analysis based on reliability of amplification and scoring (Table 1). All samples were amplified at all 13 loci using forward primers labeled with a fluorescent dye, 0.5 units Taq (GeneChoice), and reagents and conditions detailed in Table 1. Amplified PCR products were purified using ethanol precipitation and genotyped using a MegaBACE 1000 DNA Analysis System (Amersham Biosciences). Raw data were analyzed
**Table 1.** Source, amplification conditions, and repeat unit of microsatellite loci. Annealing temperatures and numbers of cycles in the PCR protocol are also presented. In loci where there are two annealing temperatures, the first was used for the first number of cycles, followed by the second number of cycles at the second temperature. Repeat unit size (Rp), focal species (the *Sebastes* species from which microsatellites were isolated), GenBank accession numbers, and reference are also shown.

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<th>Locus</th>
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<th>MgCl₂ (mM)</th>
<th>Primer (µM)</th>
<th>DNA (µL)</th>
<th>Annealing temp. (°C)</th>
<th>Cycles</th>
<th>Rp</th>
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^aL.W. Seeb et al., Alaska Department of Fish and Game.
with Genetic Profiler software (Amersham Biosciences), and automated allele assignments were manually reviewed for accuracy. DNA from 96 randomly picked samples was re-extracted and used in a blind trial to estimate genotyping error.

**Data analyses and simulations**

Genotype frequencies were tested for conformance to Hardy-Weinberg and linkage equilibrium by using the software package GENEPOP v3.4 (Raymond and Rousset 1995). Loci were also checked for evidence for mis-scoring due to large allele dropout, stuttering, and null alleles (Micro-Checker, van Oosterhout et al. 2004). Observed and expected heterozygosities, inbreeding coefficient ($F_{IS}$) values, and allelic diversities were estimated using FSTAT (Goudet 1995).

Parentage analysis was carried out based on exclusion, as well as by a method based on breeding likelihood (Sancristobal and Chevalet 1997). As the results of exclusion and likelihood were almost identical, only exclusion results are presented. Parents matching at a minimum of 11 out of 12 loci were considered in the assignment. Average exclusion probabilities (i.e., the average probability of excluding an unrelated individual as a parent) per locus and overall loci were calculated following the methods in Marshall et al. (1998).

To assess the power of the approach, simulations were carried out using the PopTools Add-In for Excel (Add-In for MS Excel, Greg Wood, CSIRO, Australia, available online at www.cse.csiro.au/poptools). First, allele frequencies estimated from all sampled fish were used to draw 137 random adults and 100 random juveniles and to estimate the number of matches that would be expected to occur by chance alone. This simulation was repeated after including 10% offspring of sampled parents to the sampled juveniles. Means and 95% confidence intervals were calculated from 1,000 permutations. Second, 10,000 random adults were drawn from the population, of which 137 were assumed to have been sampled. These 10,000 parents were used to produce 100 offspring—by chance, about 2.7% of these offspring would have at least one parent in the sample of 137 fish. Because of the large matrices involved in these simulations, means and 95% confidence limits of genotype matches were estimated from only 100 permutations. These simulations were used to estimate the distribution of the number of loci by matching between true parent-offspring pairs and between random matches. We also evaluated the difference in match between the most likely parent and the second most likely parent. In order to evaluate the significance of locus variability, we repeated the above simulations by drawing adult genotypes from allele frequencies of the four most polymorphic loci (Spi6, Sra16-5, Sal1, Sra7-25) three times. In all simulations, genotyping error was included by replacing a proportion of offspring alleles equal to the empirically estimated genotyping error with an allele randomly
drawn from the populations. Although this type of genotyping error may be somewhat unrealistic, distinction between different classes of errors is difficult and currently generally not implemented (Marshall et al. 1998, Duchesne et al. 2002). Furthermore, our mode of genotyping error is probably the most conservative in the present context, because replacing the true allele with a common allele is likely to increase the proportion of false matches.

An alternative approach to parental identification is the calculation of relatedness coefficients (Queller and Goodnight 1989). The program Kinship (Goodnight and Queller 1999) was used to simulate expected distributions of relatedness coefficients between unrelated individuals and parent offspring pairs. These distributions were compared with observed distributions of relatedness coefficients in the real data. Additionally, a log-likelihood test that compared the likelihood of unrelated vs. parent offspring relationships was carried out in Kinship—the number of tests significant at the 0.05 level was then compared within adults and recruits, and between these two groups.

**Results**

**Locus variability and genotyping error**

After repeating failed PCR amplifications, 99.2% of all genotypes were successfully determined. Variability was moderate; expected heterozygosity ranged between 0.6 and 0.9, and the mean allelic diversity was 13 alleles (Table 2). At most loci, there was no evidence for deviation of genotypic proportions from expected Hardy-Weinberg proportions—only Sra 15-8 and Sma 10 showed a significant deficiency of heterozygotes in both adults and recruits, and there was a significant excess of heterozygote adults at Sme 5. Correspondingly, Micro-Checker provided no significant evidence for mis-scoring due to stuttering or large allele drop out at most loci, with the exception of Sma 10, where there was significant evidence for mis-scoring due to stuttering. The genotyping error varied widely between loci, with no detectable error in 96 samples in seven of the thirteen loci, and an error of more than 7% at Sma 10. Because of the high genotyping error and the evidence for scoring problems, Sma 10 was excluded from subsequent analyses.

Out of the 66 possible tests for deviations from linkage equilibrium, eleven were significant at the 0.05 level in the adults, and five tests were significant in the recruits. There was no correspondence in apparent linkage between adults and recruits, except for the tests between Spi 4 and Spi 6. It is unlikely that loci other than Spi 4 and Spi 6 were indeed physically linked in a genome, a consideration that is important for the calculation of exclusion probabilities.

As expected from the relatively low variability estimates, exclusion probabilities (that is, average probabilities of excluding a randomly
Table 2. Vital statistics of loci surveyed in potential parents (A) and recruits (R), including heterozygosities (observed $H_O$ and expected $H_E$), inbreeding coefficient ($F_{IS}$) and significant deviation from Hardy-Weinberg equilibrium, and allelic diversity ($NA$ adjusted to a sample size of 135). An estimate of genotyping error based on a blind repeat screening of 96 random samples is also shown. Exclusion probabilities are based on allele frequencies from all fish. Bold $F_{IS}$ values indicate significant deviations of genotype distributions from Hardy-Weinberg equilibrium with a $P < 0.05$; bold underline with a $P < 0.01$.

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<th>$H_E$</th>
<th>$F_{IS}$</th>
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<th>$H_O$</th>
<th>$H_E$</th>
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chosen individual from parentage) were also low (Table 2). Nevertheless, over all loci the exclusion probability was 99.88%.

**Parental assignment**

Of the 209 offspring, 25 matched at least one parent at all 12 loci, and 99 offspring matched at least one parent at 11 loci. Therefore, allowing for genotyping error at one locus, more than half of all offspring had at least one potential parent in the sample, despite the high average exclusion probability. Simulations showed that this distribution of matches is very close to that expected from chance matches among random genotypes, but that it is significantly lower than expected if 10% of juveniles are offspring of sampled adults (Fig. 1). These results indicated that true offspring of resident parents, if present, were relatively rare (<10% of juveniles).

The notion of rarity of true offspring was confirmed by relatedness indices. Both pairwise relatedness between adults and between adults and recruits closely followed the distribution of relatedness
indices expected for unrelated fish (Fig. 2). However, there were some indications for relatedness from the number of log-likelihood tests that showed a significantly higher likelihood ($P < 0.05$) of first order relatedness as compared to the null hypothesis of no relatedness. Of the pairwise tests between adults, 5.2% were significant, while 6.0% were significant between adults and recruits. This slight excess of significant tests above the expected 5.0% may suggest a few, but not many, related individuals.

Simulations with 10,000 candidate parents, of which 137 parents were known, confirmed that about 10% of juveniles without a parent in the sample would have a random match at all 12 loci with one of the sampled adults (Fig. 3A). Although most juveniles with a sampled parent would also show such a match, they would not be obviously distinguishable from random matches. Furthermore, about 10% of the offspring with parents would match their true parent at only 11 loci because of genotyping error. It may be that a better distinguishing feature between true and random matches is the difference in the number of parents matching equally well. However, although the majority of offspring with parents in the sample have only their parent matching at
Figure 3. Results from simulations assuming a sample of 137 fish from a population of 10,000 candidate parents. Juveniles with parents in the sample are shown in dark gray and on the right hand scale, whereas juveniles with unsampled parents are shown in light gray and on the left hand scale. Error bars show confidence limits from 100 simulations. (A) Distribution of best matches between adults and juveniles using the original set of loci. (B) Distribution of the number of adults matching offspring at as many loci as the best matching candidate parent. (C) and (D): As in (A) and (B), but using the four most polymorphic loci three times.
all 12 loci, about 40% of random matches also have only one adult that matches best (Fig. 3B). Having a single matching parent is therefore no indication of a true parent-offspring relationship.

Equivalent simulations using the four most polymorphic loci taken three times demonstrated the value of a highly polymorphic data set for parental identification. Most offspring with no parent in the sample match any parent by chance at only five loci, and only 0.2% match at all 12 loci (Fig. 3C). In contrast, 96% of offspring with parent in the sample match that parent at all 12 loci—the other 4% being due to genotyping error. Interestingly, however, the number of potential parents matching at the same number as the maximum match was still not a good indicator for true parentage, as in almost 60% of random matches there was a single sampled parent matching best.

**Discussion**

The results of this study suggested that the molecular markers used here were not sufficiently variable to provide an unambiguous identification of offspring of resident rockfish. However, the data did indicate very limited self-recruitment, and in conjunction with SrCl$_2$ marking, led to the identification of a single offspring of an injected fish (Buckley et al. 2007). Furthermore, simulations showed that a more polymorphic marker set would have the power to identify offspring, despite the large number of potential candidate parents and the very small proportion of parents sampled.

**Parental assignments and rates of self-recruitment**

Our study identified 124 potential offspring of the rockfish sampled (matching at 11 or 12 loci to any candidate parent). However, simulations suggested that a very similar number of matching offspring would be expected by chance alone. Simulations that included 10% “true” parent-offspring pairs produced significantly more matching pairs than observed, which suggested that the true value of self-recruitment is less than 10%. On the other hand, SrCl$_2$ marking clearly identified one offspring of an injected female (Buckley et al. 2007). Therefore, genetic and otolith results combined suggested that self-recruitment did occur, but at a rate of less than 10%, though any such conclusions are very preliminary due to the lack of accurate confidence estimates.

Low levels of self-recruitment may be expected, because of the long larval period of brown rockfish (2-2.5 months, Love et al. 2002) and the consequent potential of extensive larval dispersal. However, several lines of evidence also suggest considerable potential for larval retention and limited realized dispersal of brown rockfish. Puget Sound is an enclosed estuary that includes several basins. The artificial reef on Point Heyer is situated in the central basin, which is connected to
northern Puget Sound via Admiralty Inlet (maximum depth 65 m) and to the southern basin via Tacoma Narrows (maximum depth 45 m) (Burns 1985). General circulation patterns are primarily caused by tidal exchange, and consist of outflow through Admiralty Inlet in the upper layer and inflow of marine waters at depth (Stout et al. 2001). In the southern part of the basin, currents generally flow north on the west side of Vashon Island, and south on the east side where Point Heyer is located (Ebbesmeyer et al. 1984). Such oceanographic patterns, bathymetric isolation, and tidal reversal of currents provide ample opportunity for larval retention via mechanisms such as vertical migration, habitat selection, and hydrographic retention (Joyeux 2001, Bilton et al. 2002). Nevertheless, on a very small scale, dispersal would probably be sufficient to explain the apparent low levels of self-recruitment on Point Heyer. Furthermore, the level of interannual variability in circulation patterns is currently understudied, though major circulation patterns, especially the depth of inflowing and outflowing currents, appear to be greatly influenced by decadal climate regimes (Ebbesmeyer et al. 1998). Recruitment estimates from additional years are therefore needed for more long term self-recruitment rates relevant to MPA design.

Expectations of self-recruitment also stem from indirect estimates of dispersal distances in brown rockfish and other *Sebastes* species. Isolation by distance patterns from microsatellite studies on the Pacific east coast indicated mean dispersal distances of less than 10 km in grass rockfish (*Sebastes rastrelliger*, Buonaccorsi et al. 2004), copper rockfish (*S. caurinus*, Buonaccorsi et al. 2002) and brown rockfish (*S. auriculatus*, Buonaccorsi et al. 2005). By assuming that the distribution of individual dispersal distances follows an exponential distribution (Botsford et al. 2001), about 10% of recruits would be expected to settle within 1 km of the point of release, that is, potentially on the same reef. These estimates correspond to our initial results, though more powerful markers are needed to confirm this notion.

An alternative explanation for the low self-recruitment rates estimated here may be a large variance in reproductive success among adults, resulting in a very limited number of adults actually producing recruits. Population genetic studies indicate that in many marine species the effective genetic population size ($N_e$) may be orders of magnitude smaller than the census population size (e.g., Turner et al. 2002, Hauser et al. 2002, Hoarau et al. 2005), which suggests that only one in several thousand fish is successful in reproducing. Maternal effects that lead to much higher larval survival in offspring of older females, in addition to the inherently higher fecundity of older and larger fish (Berkeley et al. 2004), may exacerbate these effects. If such few reproducitively successful adults were not included in the adult sample, their offspring might not be identified, and self-recruitment rates would be underestimated. Indeed, most females sampled were smaller than 30
cm and inclusion of the few larger fish on the reef may increase the self-recruitment estimate considerably.

Variation in reproductive success may occur either among individuals within subpopulations (Hauser et al. 2002) or among subpopulations (Turner et al. 2002). Indeed, variation in productivity among subpopulations may decrease $N_e$ by several orders of magnitude (Turner et al. 2002). Because self-recruitment was estimated as a proportion, it does not only depend on the productivity of the Point Heyer population, but also other populations in the vicinity, and may have been reduced by immigration from very productive populations. Such considerations emphasize the importance of interannual variability in recruitment.

Methodological considerations

Parentage analysis of marine species is not a trivial undertaking. “Biological” assumptions of common parentage methods are that the population is fairly small, that a large proportion of candidate parents have been sampled, that both parents are in the sample, and that the juvenile sample contains a large proportion of offspring of known parents. Violations of these assumptions result in a progressively restrictive range of analysis methods (Jones and Ardren 2003). The population size of brown rockfish in Puget Sound has been estimated as 100,000 individuals (Stout et al. 2001), all of which are potential parents of juveniles recruiting to the Point Heyer reef. However, the adult population of brown rockfish at Point Heyer is 200-250 fish (Buckley et al. 2007) and so about half of the adults were included in this study ($N = 137$). There is therefore a $25\%$ chance that any true offspring of resident adults has both parents in the sample—a probability that is lower than in most other studies of parentage assignment (Jones and Ardren 2001). The proportion of offspring of these adults in the recruiting juveniles is unknown, and may range from 0% given the extensive larval period of 2 to 2.5 months (Love 2002) to 15-60% as was reported for reef fish (*Pomacentrus amboinensis*, Jones et al. 1999) on the Great Barrier reef. All these ecological features of many marine species, such as brown rockfish, complicate parental assignment, but certainly should not prevent it, given the appropriate set of marker loci and some way of verifying assignments.

There are also some assumptions regarding the markers in question. Most importantly, markers should have high variability, and so provide high exclusion probability for random genotypes. Our marker set was less variable than one would like for a parentage assignment study. Two reasons may account for this low variability: first, all loci were isolated from other rockfish species, and it is known that microsatellites are somewhat longer and more variable in the focal species (the species from which it was isolated) than in related species (Neff and Gross 2001). Second, brown rockfish in Puget Sound have lower genetic diversity
than their conspecifics on the Oregon and California coast (Buonaccorsi et al. 2005). The relatively low variability of microsatellites led to low exclusion probabilities (0.2-0.7, Table 2) and further reduced the power of parentage assignment.

Nevertheless, our simulations showed clearly that a more variable microsatellite data set would have high power in detecting true parent-offspring pairs in our samples. Using our suite of loci, about 10% of random pairs of individuals matched at all 12 loci, whereas a more variable set of microsatellites would reduce this percentage to 0.2%, while leaving the distribution of matches between true parent-offspring pairs unchanged. Similar increases in parentage assignment success with increasing diversity of marker loci were observed in other simulation studies (Bernatchez and Duchesne 2000). Interestingly, the distribution of numbers of candidate parents matching the offspring at the same number of loci as the maximum match found did not change noticeably with marker variability. This number of equally well matching parents is closely related to the concept of the difference in match between the most likely parent and the second most likely parent, a criterion often used in parental assignment studies (Marshall et al. 1998). These results suggest that with a small sample from a large number of candidate parents and genotyping error, such a criterion may be unsuitable.

Low genotyping error and low mutation rates are a further assumption of parental assignment. Some approaches allow the consideration of genotyping errors (e.g., Marshall et al. 1998), but in doing so reduce the power of assignment even further. Simulations that used a maximum likelihood approach (Marshall et al. 1998) showed that low marker variability, genotyping error, and a large proportion of unsampled parents together reduce the power sufficiently to prevent confident parental assignment (data not shown). In our study, the locus with the highest genotyping error (Sma 10) was removed from the analysis, and the remaining average genotyping error (1%) was within the average commonly observed in microsatellite studies (Hoffman and Amos 2005). Nevertheless, over the 12 loci, the genotyping error exceeded 12% per multilocus genotype, which resulted in false exclusion of parent-offspring pairs. Furthermore, because of the rapidly increasing multilocus genotyping error, the addition of more loci does not always result in higher power of parental assignment, as the limited increase in information content with each additional locus is more than offset by an increase in overall error rate. It would therefore be best to use a limited number of loci with high variability and low genotyping error for parental assignment.
Implication for MPA design

One of the main assertions of MPAs is that protecting a spawning biomass of marine fishes will enhance progeny output, and that the patterns of larval dispersal from the MPAs will contribute to local and/or regional stocks. However, the dispersal of marine fish larvae is poorly documented and this basic assumption remains unverified. Although clearly only a “snapshot” in time, our results suggested that, at least on Point Heyer, recruitment of the 2004 year class occurred primarily from outside the resident population. However, concluding a low conservation priority from these data would be misleading, because data from additional years are needed to establish long-term temporal trends. Furthermore, the destination of Point Heyer offspring is currently unknown, and the population may be an important source of larvae for other reefs. Point Heyer may thus be an important component of the rockfish habitat in Puget Sound. Further surveys including other reefs are needed, optimally using a combination of otolith marking techniques (Buckley et al. 2007) and genetic methods. Our simulations clearly demonstrated the feasibility of the approach of identifying offspring of resident adults, which may be a powerful tool for estimating dispersal into and out of MPAs.

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References


