

Population size estimates for the tidepool sculpin, (*Oligocottus maculosus*) and resulting genetic implications for San Juan Island populations

Jackie Carter
Friday Harbor Laboratories
Marine Fish Ecology
Spring 2004

Population size estimates for the tidepool sculpin, (*Oligocottus maculosus*) and resulting genetic implications for San Juan Island populations

Jackie Carter
Marine Fish Ecology
Friday Harbor Labs
Spring 2004

Abstract

The tidepool sculpin is a small intertidal fish with limited larval dispersal, homing capabilities, and a sedentary adult stage, that make it an ideal candidate for a genetic differentiation study. Previous work found genetic differences between two sites along the coast of San Juan Island. We estimated population sizes at these sites and others to potentially offer an explanation for the differentiation. Population size estimates were found to be highly variable between sites, ranging from 89 to 654 fish. A collaborator performed further genetic analysis of the fish at these sites and found no differentiation. The potential mechanisms behind both genetic findings are discussed and put into a population size context.

Introduction

The tidepool sculpin, *Oligocottus maculosus*, is a demersal species found in the intertidal zone, particularly in tidepools and rocky reefs (Green 1971a, Green 1971b, Green 1973, Nakamura 1976, Pfister 1995, Pfister 1996, and Yoshiyama et al. 1992). It lacks an air bladder (Moring 2001), making it well suited to reside at the bottom of the pool, a habitat selection that results in protection from the turbulent intertidal environment above. The tidepool sculpin prefers pools in the mid- to upper- intertidal zone (Nakamura 1976, Pierce and Pierson 1990), and other studies have found larger fish in the lower part of their intertidal range (Green 1971, Pierce and Pierson 1990, Szabo 2002). In addition to exhibiting vertical zonation, it has been shown that the abundance of this particular species is correlated with pool volume (Pfister 1998). Like most intertidal fish, the tidepool sculpin has a very high tolerance to changes in temperature

(Moring 2001 and Nakamura 1976) and salinity (Nakamura 1976) and inhabits pools of highly variable physical and chemical characteristics (Nakamura 1976). It lays demersal eggs and larvae are pelagic (Horn et al. 1999). Generally, pelagic larvae are at the mercy of the currents, but these tend to remain in the nearshore waters adjacent to where they were spawned (Marliave 1986, Yoshiyama and Sassaman 1993). Juveniles settle in the upper intertidal zone (Craik 1980), although it is not clear exactly when settlement occurs. Recent evidence suggests that this species spawns year-round and that settlement is continuous (Lyle Britt, *personal communication*, 2004).

The focus of a number of studies has been the demonstration of high site fidelity among juveniles and adults (Altman and Taylor 2003, Eschmeyer and Herald 1983, Green 1971*a*, Green 1973, Moring 2001, Yoshiyama et al. 1992). Additionally, many transplant experiments have shown that *O. maculosus* has the ability to return to its original tidepool from distances exceeding 100m (Altman and Taylor 2003, Eschmeyer and Herald 1983, Green 1971*a*, Green 1973, Moring 2001, and Yoshiyama et al. 1992), although the time scale varies both within and among populations (Moring 2001). This process, known as homing, is moderated by olfaction rather than vision (Khoo 1971). It also appears that larger, presumably older, fish are more capable of homing than their smaller conspecifics, but that homing ability declines in the largest size class (Craik 1981). Craik (1981) also noted that juveniles show extensive movement between pools before finally choosing a home range and demonstrating homing behavior. This more pronounced movement might also be partially attributed to competitive exclusion by older, larger conspecifics (Szabo 2002).

The tidepool sculpin makes a particularly good candidate for genetic sub-structure analysis due to life history characteristics mentioned above, especially demersal eggs, limited larval dispersal, homing capabilities, small size, and short generation time (Hellberg et al. 2002). As a result of demonstrated homing behavior, individuals generally remain in the same area throughout their lives, and, because of their size, have reduced potential for adult dispersal (Green 1971*b*). Homing capabilities have been shown to promote reproductive isolation and genetic structuring between populations (Ramstad et al. 2004). The demonstrated reduced larval dispersal of the tidepool sculpin (Marliave 1986) may also lead to closed populations. In other words, there may be no migrants between populations, hence no gene flow. Genetic markers can be used as an indication of closed populations because genetic differentiation is highly sensitive to migration (Hellberg et al. 2002).

A number of studies have addressed the issue of population sub-structure in the tidepool sculpin using molecular markers (Altman and Taylor 2003, Caponera and Brockovich 2003, Yoshiyama and Sassaman 1993). Altman and Taylor (2003) analyzed mitochondrial DNA (mtDNA) from populations on southwestern Vancouver Island, British Columbia and northeastern Vancouver Island and found no genetic variation between the two areas, but did find variation among tidepools within sites. Likewise, Yoshiyama and Sassaman (1987) found no inter-locality differences in allozyme variation among three species of tidepool-residing sculpin, including *O. maculosus*. However, Caponera and Brockovich (2003, *unpublished student paper*, FHL) analyzed mtDNA and microsatellites and found small-scale (<20km) genetic differentiation in

tidepool sculpin populations between sites along the southwest coast of San Juan Island, Washington. It is from this finding that the current study stems.

The question remains as to why there is differentiation between these sites. Are there any specific characteristics of these populations that would promote one mechanism of differentiation over another? One hypothesis is that the individual populations are small and drift is the driving force behind the variation. Genetic drift affects finite populations and is the random change in allele frequencies due to chance sampling events (Herron and Freeman 2001). The effects of drift hinge upon the effective population size (N_e), or the number of individuals that contribute genetically to the next generation (Hellberg et al. 2002). In marine species, variable spawning success and pelagic larval survival may translate to an N_e which is much lower than the actual census size (Hellberg et al. 2002). Genetic drift promotes divergence between sites, an effect that may be compounded if population sizes, and subsequently N_e , are small.

The differences found by Caponera and Brockovich (2003) also raise many questions about the mechanisms of gene flow between populations of the tidepool sculpin along the southwest coast of San Juan Island. Because only two sites along the west coast were examined, it is unclear at this point which gene-flow models are appropriate to explain variation along the coast. While most patterns of genetic variation can be accounted for by selection for different alleles in different populations due to varying environmental factors (Hellberg et al. 2002), the stepping stone modification of the isolation by distance model (Kimura and Weiss 1964) may best explain the possible patterns of differentiation between areas once fish from more sites are examined.

Isolation by distance assumes a gradual increase in variation with an increase in distance (Van der Strate et al. 2003). The stepping stone modification of this model proposes that individuals are dispersed discontinuously to form many colonies and may be exchanged between colonies from adjacent colonies (Kimura and Weiss 1964). For example, fish from Cattle Point may migrate only as far as Eagle Cove, while Eagle Cove fish may migrate to Cattle Point or False Bay and False Bay fish may move between Eagle Cove or Deadman Bay (Fig. 1). If this is the case, with further analysis we would expect to see a gradient of differentiation along the southwest coast of San Juan Island.

The goal of the present study was to estimate the size of tidepool sculpin populations at three sites along the southwest coast of San Juan Island, and, in conjunction with the results of a genetic substructure analysis of these populations (see Dwyer 2004, *unpublished student paper*, FHL), provide an explanation of the potential mechanisms driving the differentiation between populations found by Caponera and Brockovich (*unpublished student paper*, FHL, 2003). We sampled both tidepool and non-tidepool habitats for *O. maculosus*, estimated population size with a mark-recapture technique, and related the conclusions to the results of the genetic analysis.

Methods

Sampling Locations

All mark-recapture studies of the tidepool sculpin (*Oligocottus maculosus*) were conducted in the San Juan Islands in Northwestern Washington. We selected three tidepool sites on the west-southwest side of San Juan Island: Cattle Pass, Eagle Cove, and Deadman Bay. Previous studies involving *O. maculosus* have focused on these sites and others in the vicinity, indicating that the species is present in most areas around the San

Juan Islands with suitable habitat. In addition to the rocky intertidal habitat, we sampled nearby non-tidepool habitat (sandy or cobble beach) to account for all fish in the area.

Field Methods

Tidepool selection: Because of time constraints, tidepools were opportunistically selected based on actual observation of *O. maculosus* in the pool or presence of habitat characteristics known to be favored by the species, such as substantial rock cover, adequate depth, and few invertebrates. The height of each pool was measured using an extended meter stick held at water level and a scope with an internal carpenter's level set on a meter stick at the edge of the pool. Surface area was estimated by taking the length and width of pools. Three depth measurements were averaged and combined with the surface area estimate to estimate the volume of the pool. Distances between pools were estimated with GPS coordinates (in decimal degrees) and a conversion to meters.

Tidepool fish collection: Tidepools were drained with a bilge pump and fishes were collected after adding a small amount of Quinaldine, an anesthetic that calms the fish (see Pfister 1996). Previous studies indicate no detrimental effects on the fish due to repeated exposure to Quinaldine (Cross 1981). Pools were then thoroughly searched until most fish were removed by dip-netting. All fish collected were immediately placed in a bucket with clean water to recover. Tidepool sculpin were identified and marked.

Non-tidepool fish collection: Non-tidepool, cobble or sandy beach habitat was sampled using a pole seine (10m x 2.75m, 2cm mesh size) and modified baited minnow traps. Seining occurred 30 minutes before and 30 minutes after low tide. Window screen was hot glued to the inside of the minnow trap and the entry holes were made smaller with fishing line to prevent escapement of fish.

Marking: There were two marking events. The first set of tidepools were assigned numbers between one and six depending on the site. The second set of pools were numbered between seven and fourteen. All tidepool sculpin were marked with a fin-clip and length, weight, and sex data were taken. Fish marked the first sampling day received a diagonal clip on the top of the caudal fin. All fish caught on the second day were examined for previous clips. Those that were not clipped received a diagonal clip on the bottom of the caudal fin. In addition to allowing us to recognize previously captured fish, marking by clipping different parts of the caudal fin facilitated a rough estimate of movement between pools, and also provided a tissue sample for genetic analysis.

Recapture: At Deadman Bay, twelve of the fifteen original pools were re-sampled for marked fish. Three new pools were also selected and sampled. Six of the original ten pools at Cattle Point were selected for recapture, plus four new pools. Seven of ten original pools at Eagle Cove were sampled, and no new pools were selected. Recapture techniques in the tidepools were the same as those used to mark the fish originally. Length, weight, and sex were recorded for captured fish. No non-tidepool-residing tidepool sculpin were captured initially, so we did not re-sample those areas.

During either the mark or recapture process, we sampled most of the pools at all sites that appeared to offer suitable tidepool sculpin habitat.

Statistical Analysis

Population estimate: The Petersen method was used to estimate the population size (N) at all three sites (Ricker 1975). This statistic assumes that fish are marked only once and a single sample is taken for recapture. Because we sampled different sets of

pools each time out, we can treat our data as a single marking event. The statistic is as follows:

$$N = \frac{(n_1 * n_2)}{m}$$

where n_1 = number of fish marked

n_2 = sample taken for census

m = number of recaptured marks in the sample

The fraction of marked fish in the second sample (n_2) should equal the fraction of the population that is marked (Pine et al. 2003). Variances were calculated using the formula

$$V(N) = \frac{n_1^2 n_2 (n_2 - m)}{m^3}$$

Results

Population estimate

Estimates of population sizes were highly variable between sites. The population at Deadman Bay was 654 fish with a lower limit of 543 and an upper limit of 747 fish. The estimated population size at Eagle Cove was much smaller, calculated to be 89 fish, with lower and upper limits of 61 and 117 fish, respectively. At Cattle Point, the population was estimated at 274, with a lower limit of 115 and an upper limit of 394 fish (Table 1).

Movement

Recapture data from Cattle Point and Eagle Cove indicate no movement of fish between pools marked with a top clip and those marked with a bottom clip. At Deadman Bay there was distinct indication of movement between pools. During recapture we found ten top clipped fish (11.9% of marked sample) in bottom clipped pools, as well as one top-clipped fish (1.19% of marked fish) in a new pool.

Distances Between Pools

The maximum distance between a top and bottom clipped pool at Deadman Bay was 55.8m, and the minimum distance was 4.1m. The maximum distance between a clipped pool and new pool was 46.3m and the minimum was 6.1m at Deadman Bay. At the east side of Eagle Cove the maximum distance between top and bottom clipped pools was 30.2m and the minimum distance was 15.9m. The maximum top-bottom clipped distance for the west side of Eagle Cove was 97.7m and the minimum was 28.8m. There were no new pools sampled at this site. At Cattle Point, the maximum distance between top and bottom clipped pools was 230.1m, while the minimum distance was 9.6m. At this site, the maximum distance between a clipped pool and a new pool was 251.8m and the minimum was 3.2m (Table 2).

Discussion

To get the best estimate of population size at each site we should have sampled all pools, suitable or not. However, because of the time constraints inherent in a ten-week study, this was impossible. Additionally, inconsistencies between sites in our recapture methodology, such as not accounting for possible movement at Eagle Cove by not sampling new pools, led to questions about what we were actually estimating. Rather

than estimating population size at the site, we may have been estimating only the population among the pools we sampled. However, indications of movement between pools can be used to clarify what we were estimating. It is established in the literature that tidepool sculpins move between more than one pool in their home range (Nakamura 1976, Yoshiyama et al. 1992). If fish showed movement between pools, we would be estimating the population size at the site. If they did not move, we would be estimating the population using the pools we sampled. The only site at which there was evidence of movement was Deadman Bay, where we recaptured ten top-clipped fish in bottom-clipped pools. These fish could have moved (from a top-clipped pool to a bottom-clipped pool) anywhere in between 4.1m and 55.8m. The single fish captured in a 'new pool' during the recapture process could have moved between 6.1m and 46.3m. This information can be used to estimate potential movement at other sites. Since the fish could potentially have moved 55.8m at Deadman Bay, we can assume that fish at Eagle Cove or Cattle Point would be equally likely to move 55.8m. If the minimum distance between pools at Eagle Cove and Cattle Point was less than 55.8m, we can infer that movement between pools might have occurred and assume that we estimated the total population at these sites.

While tidepool sculpins have been shown to move over 100m to return to their home range of pools after transplantation experiments (Cross 1981, Eschmeyer and Herald 1983), it is much more likely that our fish moved only the minimum distance between a top- and bottom- clipped pool or between a clipped pool and a new pool. Regardless of this fact, I feel confident that we estimated the total population and each

site because we did sample most, if not all, of the pools offering suitable tidepool sculpin habitat.

Results of the genetic analysis indicate no genetic differentiation between populations of tidepool sculpin along the southwest coast of San Juan Island (Dwyer 2004, *unpublished student paper*, FHL). Our original hypothesis to explain the differentiation found by Caponera and Brockovich (2003, *unpublished student paper*, FHL) was that the populations were small and their genetic features were governed by drift. It appears now that this may not necessarily be the case. While the population sizes may be considered small, and effective population sizes (Herron and Freeman 2001) are much smaller (Deadman Bay $N_e=378$, Eagle Cove $N_e=62$, Cattle Point $N_e=96$), by implication, drift may be playing an important role in the genetic dynamics. However, the lack of population substructure indicates that there is enough gene flow to prevent population substructure. Larval transport may be the best explanation of this phenomenon.

It is known that tidepool sculpin larvae remain nearshore, actively swim against the current, and aggregate to protected areas (Marliave 1986, Shanks et al. 2003). For these reasons, it is an accepted theory that there is very little larval dispersal in this species, hence very little gene flow. However, even with very localized gene flow via larval dispersal, it is still possible that stochastic oceanographic events can cause long distance larval dispersal (Yund and O'Neil 2000), and potentially adult dispersal (Altman and Taylor 2003). These chance occurrences may provide sufficient gene flow along the west coast of San Juan Island despite low rates of active larval dispersal and sedentary

adult life history characteristics (Altman and Taylor 2003). This is likely the reason we see no step-wise gradient of allele frequencies along the island.

To fully assess the behavior of these populations of tidepool sculpin, further analysis is needed. This collaborative study addressed the issues of population size and the genetic implications of those findings, genetic differentiation (or lack thereof) between populations on San Juan Island (Dwyer 2004, *unpublished student paper*, FHL), and possible ecological causation of varying population sizes (Kiyohara 2004, *unpublished student paper*, FHL). More thorough and consistent sampling techniques, as well as more genetic samples, would add depth to the information gathered throughout the course of this study.

Acknowledgements

First and foremost, I would like to thank my study partners, Kelly Kiyohara - for the time and effort she put into our project, and Tim Dwyer – for all his help, both in the field and lab. I would also like to thank Drs. Don Gunderson and Art Kendall, and our TAs, Nick Lowry, Beth Matta, and Anne Beaudreau for their advice and guidance throughout this process, as well as Lyle Britt for giving us the background information that inspired the study. A special thanks goes out to everyone who helped with fish collection: Mia Norheim, Kristin Hannan, Kathy Ireland, Marina Tagliaferro, Beth Matta, Anne Beaudreau, Don Gunderson, Nick Lowry, Josh Chamberlain, Mike Baltzley, Heather Baltzley, and any others I may have left out. Without the financial support of the Washington Research Foundation, Howard Hughes Medical Institute, ARCS Foundation (Achievement Rewards for College Scientists), Friday Harbor Laboratories' Discretionary Fund for Excellence, Mary Gates Scholarship Foundation and the Office of the Provost, University of Washington, this work would not have been possible. Last, but not least, thank you to the Marine Fish Ecology apprenticeship team and the rest of the lab community for making these past ten weeks an amazing experience.

Works Cited

- Altman, A.D. and E.B. Taylor. 2003. A molecular assessment of homing in the tidepool sculpin. *J. Fish Biology*. **62**: 623-640.
- Caponera, J. A. & Brokovich, E. 2003. Limited dispersal in the intertidal fish *Oligocottus maculosus*, and its implication for marine reserve design (unpublished student paper – Friday Harbor Laboratories).
- Craik, G. J. S. 1980. The effects of age and length on homing performance in the intertidal cottid, *Oligocottus maculosus*. *Canadian Journal of Zoology*. **59**: 598-604.
- Cross, J.N. 1981. Structure of a rocky intertidal fish assemblage. PhD Thesis, University of Washington.
- Dwyer, T. 2004. Population genetic subdivision in the tidepool sculpin *Oligocottus maculosus* in the San Juan Islands, Washington. Unpublished student paper, Friday Harbor Laboratories.
- Eschmeyer, W.N., and E.S. Herald. 1983. A Field Guide to the Pacific Coast Fishes. Houghton Mifflin Company: Boston pp. 178.
- Green, J.M. 1971a. High tide movements and the homing behaviour of the Tidepool Sculpin, *Oligocottus maculosus*. *Journal of the Fisheries Research Board of Canada*. **28**: 383-389.
- Green, J.M. 1971b. Local distribution of *Oligocottus maculosus* Girard and other tidepool cottids of the west coast of Vancouver Island, British Columbia. *Canadian Journal of Zoology*. **49**: 1111-1128.
- Green, J.M. 1973. Evidence of homing in the Mosshead Sculpin (*Clinocottus globiceps*). *Journal of the Fisheries Research Board of Canada*. **30**:129-130.
- Hellberg, M.E., R.S. Burton, J.E. Neigel, and S.R. Palumbi. 2002. Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science*. **70**(supplement): 273-290.
- Herron, J.C. and S. Freeman. 2001. *Evolutionary Analysis*. Prentice Hall: Upper Saddle River, NJ.
- Horn, M.H., K.L.M. Martin, and M.A. Chotkowski. 1999. *Intertidal fishes – Life in two worlds*. Academic Press: San Diego.
- Kimura, M.,G.H. Weiss. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*. **49**: 561-576.

- Kiyohara, K. 2004. *Oligocottus maculosus*: A habitat analysis for three sites on San Juan Island, Washington. Unpublished student paper, Friday Harbor Laboratories.
- Khoo, H.W. 1974. Sensory basis of homing in the intertidal fish *Oligocottus maculosus* Girard. *Canadian Journal of Zoology*. **52**: 1023-1029.
- Marliave, J.B. 1986. Lack of planktonic dispersal of rocky intertidal fish larvae. *Transactions of the American Fisheries Society*. **115**: 149-154.
- Moring, J.R. 2001. Appearance and possible homing of two species of sculpins in Maine tidepools. *Northeastern Naturalist*. **82**: 207-218.
- Nakamura, R. 1976. Temperature and the vertical distribution of two tidepool fishes (*Oligocottus maculosus*, *O. snyderi*). *Copeia*. **1**: 143-152.
- Pfister, C.A. 1995. Estimating competition coefficients from census data: A test with field manipulations of tidepool fishes. *American Naturalist*. **146**: 271-291.
- Pfister, C.A. 1996. The role and importance of recruitment variability to a guild of tidepool fishes. *Ecology*. **77**: 1928-1941.
- Pfister, C.A. 1998. Extinction, colonization, and species occupancy in tidepool fishes. *Oecologia*. **114**: 118-126.
- Pierce, B.E. and K.B. Pierson. 1990. Growth and reproduction of the tidepool sculpin, *Oligocottus maculosus*. *Japanese Journal of Ichthyology*. **36**: 410-417.
- Pine, W.E, K.H. Pollock, J.E. Hightower, T.J. Kwak, J.A. Rice. 2003. A review of tagging methods for estimating fish population size and components of mortality. *Fisheries*. **28**:10-23.
- Ramstad, K.M, C.A. Woody, G.K. Sage, F.W. Allendorf. 2004. Founding events influence genetic population structure of sockeye salmon (*Oncorhynchus nerka*) in Lake Clark, Alaska. *Molecular Ecology*. **13**: 277-290.
- Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada*. **191**: 77-78.
- Shanks, A.L., B.A. Grantham, M.H. Carr. 2003. Propagule dispersal distance and the size and spacing of marine reserves. *Ecological Applications*. Supplement **13**: 159-169.
- Szabo, A.R. 2002. Experimental tests of intercohort competition for food and cover in the tidepool sculpin (*Oligocottus maculosus* Girard). *Canadian Journal of Zoology*. **80**:137-144.

- Van der Strate, H.J., L. Van de Zande, W.T. Stam, R.J. Haroun, J.L. Olsen. 2003. Within-island differentiation and between-island homogeneity: non-equilibrium population structure in the seaweed *Cladophoropsis membranacea* (Chlorophyta) in the Canary Islands. *European Journal of Phycology*. **38**: 15-23.
- Yoshiyama, R.M., K.B. Gaylord, M.T. Philippart, T.R. Moore, J.R. Jordon, C.C. Coon, L.L. Schalk, C.J. Valpay, and I. Tosques. 1992. Homing behavior and site fidelity in intertidal sculpins (Pisces: Cottidae). *Journal of Experimental Marine Biology and Ecology*. **160**:115-130.
- Yoshiyama, R.M. & C. Sassaman. 1987. Geographical patterns of allozymic variation in three species of intertidal sculpins. *Environmental Biology of Fishes*. **21**: 203-218.
- Yoshiyama, R. M. & C. Sassaman. 1993. Levels of genetic variability in sculpins (Cottidae: Teleostei) of the North American Pacific coast and an assessment of potential correlates. *Biological Journal of the Linnean Society*. **50**: 275-294.
- Yund, P.O. and P.G. O'Neil. 2000, Microgeographic genetic differentiation in a clonal ascidian (*Botryllus schlosseri*) population. *Marine Biology*. **137**: 583-588.

Tables and Figures

Table 1. Estimation of population sizes at three sites along the southwest coast of San Juan Island. Variances and upper and lower limits are indicated.

	N	Variance	Upper	Lower
Deadman Bay	654	2874.68	747	543
Eagle Cove	89	219.98	117	61
Cattle Point	274	3955.04	394	155

Table 2. Maximum and minimum distances in meters between top-clipped pools and bottom-clipped pools, as well as maximum and minimum distances between pools with a clip and pools selected on the recapture day (t=top, b=bottom, c=clipped, n=new pool).

Deadman Bay	max t-b 55.8	min t-b 4.1	max c-n 46.3	min c-n 6.1
Eagle Cove E	max t-b 30.2	min t-b 15.9	max c-n n/a	min c-n n/a
Eagle Cove W	max t-b 97.7	min t-b 28.8	max c-n n/a	min c-n n/a
Cattle Point	max t-b 230.1	min t-b 9.6	max c-n 251.8	min c-n 3.2

Figure 1. Map of San Juan Island and the study sites. Deadman Bay is the site furthest west, followed by Eagle Cove in the middle, and Cattle Point on the southeastern tip.

