If there is any generalization to be made about populations of commercially harvested marine species, it is that they all show dramatic numerical fluctuations in both space and time. Traditional approaches to fisheries management, based on standard stock-recruitment models, have repeatedly led to overexploitation of commercially valuable stocks throughout management history. Notable examples in California fisheries include the halibut, sardine, anchovy, salmon, and urchin fisheries. A recent survey of Dungeness crab fisheries practices and catch history suggests that this fishery is also in imminent danger of collapse (Orensanz et al. 1998). It is becoming clear that sustainable management of commercially harvested finfish and shellfish with complex life cycles must include an understanding of how patterns of larval distribution, abundance and dispersal regulate adult population dynamics. This, in turn, depends on explicit knowledge of population structure as well as patterns and amount of larval dispersal between localities. However, to date inferences of the patterns and magnitude of gene flow among populations of marine invertebrates have been “deduced only through weak inference” (Hairston 1989).

In contrast to traditional approaches of estimating larval dispersal potential, using the type of high-resolution genetic data we collected throughout the course of this project allowed us to provide the most detailed inference of larval dispersal for a marine species with long-term planktonic larvae to date. This research was the focus of RJ Toonen’s dissertation (Toonen 2001) and was pivotal in his ability to complete that project and graduate. Toonen has since gone on to a faculty position at the University of Hawaii at Manoa, and R/F-177 played an integral role in that success. In addition to Toonen’s dissertation, there are 4 publications currently published or in review (see below), and another 7 in preparation. We plan to have all these manuscripts submitted within the next year. In total R/F-177 will have contributed directly to the dissertation of one doctoral student, and will produce peer-reviewed 11 publications. In addition, it has been possible to train 5 undergraduate students (M. Locke, T. Mai, C. Louie, C. Keever & C. Wee) during the course of this funding, 3 of whom are currently enrolled in graduate school (M. Locke, T. Mai, C. Louie) and the final two students are seniors who intend to attend graduate school next year.

Understanding the role of planktonic larval dispersal remains one of the major hurdles facing marine population biologists today. In an effort to better understand patterns and magnitudes of larval dispersal in coastal marine species, we developed microsatellite (simple sequence repeat) loci for both the Dungeness crab, *Cancer magister*, and the porcelain shore crab, *Petrolisthes*
In the process of this project, we developed a new technique that allowed increased throughput for genotyping the thousands of individuals included in this study. Our improved protocol has resulted in two publications (Toonen & Hughes 2001, Toonen & Hughes 2003).

Using techniques developed in our lab (Toonen 1997), we also developed a series of 17 highly polymorphic microsatellites (up to 55 alleles per locus) for both species. We also developed species-specific primers for the cytochrome oxidase I (COI) gene in the mitochondrial DNA of each species, these primers will be included in a manuscript in preparation that compares the genetic analysis of the nuclear microsatellite markers to that obtained using an mtDNA marker. We have submitted a manuscript detailing the isolation and amplification techniques for the microsatellite loci isolated from Dungeness crab (Toonen et al. In review), and the sequences from which those primers were developed have already been submitted to GenBank (accession numbers AY359589-AY359605). Of the 17 loci developed for C. magister, six passed the quality-control screening and appeared suitable for the following analyses (as outlined in the submitted manuscript). The manuscript outlining the techniques and primers used for scoring these loci in the shore crab P. cinctipes is currently in preparation. In contrast to the case with C. magister, 15 of the 17 loci developed for P. cinctipes failed to meet the assumptions of population genetic analyses, and were therefore excluded from these analyses (Toonen 2001).

In proposing this research, we set forth the specific objective of testing whether: a) the geographic distribution represents a single panmictic population or a set of demographically independent subpopulations, b) populations south of coastal promontories (such as Pt. Arena, or Pt. Reyes) are genetically distinct from those to the north of them (as expected from inferences of larval dispersal based on physical oceanographic processes, e.g., (Wing et al. 1995a, Wing et al. 1998, Wing et al. 1995b), and c) specific populations are seeded by different source populations in space and time.

With the assistance of collaborators in Alaska (T. Shirley), British Columbia (B. Crespie), and Washington (P. Jensen), we have completed a survey of 12 sites that comprise much of the species range. Using microsatellite loci, we found evidence for slight, but statistically significant population differentiation among crab populations sampled in different states. Thus, gene flow resulting from larval dispersal appears unable to completely homogenize Dungeness populations throughout the range. Our results argue that although there appears to be some evidence for demographic independence among locations, the overall degree of population differentiation does not suggest that there are any major barriers to dispersal for Dungeness crab throughout the Pacific Northwest.

We were not able to sample Dungeness crab as intensively as we would have liked to conduct this study, and we therefore turned to a surrogate crab
species to better understand the patterns of population genetic structure observed. *P. cinctipes* is much more abundant and easily sampled at all life stages than *C. magister*. Therefore, we sampled this species intensively in each of 12 locations spanning nearly the entire species range (from Morro Bay, CA to Neah Bay, WA) in each of three consecutive years (1997, 1998 & 1999). As mentioned previously, 15 of the 17 polymorphic loci violated one of the primary assumptions of population genetic analyses (Toonen 2001); therefore, only data from the remaining two loci were used in subsequent analyses of genetic structure among sites and size classes of *P. cinctipes* in each of 1997, 1998 & 1999.

As with the Dungeness crab data above, inferred levels of effective gene flow among populations decrease with geographic distance of separation between sites. However because we have a greater sample size with this data set we were able to determine that this relationship appears nonlinear. A parabolic relationship between pairwise estimates of gene flow (M_hat) and geographic distance of separation (km) among populations of *P. cinctipes* explains 35% of the observed variation. This curvilinear relationship suggests that larvae are unlikely to settle either very close to the site of release or extremely far from it. Thus, pelagic larvae of *P. cinctipes* appear to settle with greatest frequency at intermediate distances from the natal site (Toonen & Grosberg In review).

The hypothesis that retention zones associated with headlands limit larval dispersal (Wing et al. 1995a, Wing et al. 1998, Wing et al. 1995b) cannot account for the observed pattern of genetic structure among geographic populations of *P. cinctipes* (Toonen & Grosberg In review). Crabs sampled from sites between promontories were frequently more genetic different than comparisons with sites across such points. These data argue that even if upwelling retention zones show a strong demographic effect in the timing of larval settlement (e.g., Wing et al. 1995a, Wing et al. 1998, Wing et al. 1995b), sufficient larvae escape the retention zones that there is no detectable genetic signature of these zones (Toonen & Grosberg In review).

Examining the genetic structure of recruits proved to be the most informative portion of this study. Clustering analyses suggest that recruits at each site are most similar to southern populations during the 1997-1998 El Niño seasons, and to northern populations during the 1999 La Niña. In fact, no group of recruits was ever assigned to a northern population during the 1997-1998 sampling seasons, whereas no group of recruits was assigned to a southern population during the 1999 sampling year (Toonen 2001). Recruits also show the greatest degree of annual variation in allelic frequencies and the lowest degree of spatial genetic structure across broad geographic scales. Within each sampling year, estimates of population subdivision (based on either Wright’s $F_{ST}$ or the microsatellite-specific analog, $R_{ST}$) among recruits does not differ
significantly from zero throughout the entire species range. In stark contrast, the adult structure remains unchanged from year-to-year.

Patterns of genetic structure appear to accumulate through time: recruits show no significant differentiation, whereas subadults and adults do. Annual variability among recruits appears to be damped through accumulation of multiple cohorts in subsequent age classes; the among year component of genetic variation decreases with age class (recruits < subadults < adults). Conversely, spatial genetic structure appears to accumulate through time within sites, such that spatial structure increases with age class (adults > subadults > recruits). Four independent estimates of range-wide population subdivision are significant and consistent. However, these single-year estimates appear misleading relative to the overall data set if the observed genetic variance is partitioned hierarchically by sampling year, site, and age class. In the overall hierarchical analysis in which size class, sampling year and site are considered explicitly, the degree of population subdivision among sites is zero. Rather than sites, collection year and age class appear to account for all the genetic differentiation among samples collected in each year of sampling.

These results suggest that estimates of gene flow based solely on adult sampling can be misleading relative to a complete age- and size-class structured sampling program. This result argues that evolutionary inferences drawn from single-time or single-age-class samples of *P. cinctipes* are unreliable. If this pattern proves to be a general phenomenon among marine invertebrates with planktonic larvae, studies of the genetic structure of marine organisms that sample populations only once through time should be viewed with caution.

These data are consistent with genetic structure in both *C. magister* and *P. cinctipes* being a combined result of 1) variation in the source of larval recruits across years, 2) individual variation in reproductive success, and 3) temporally and spatially variable natural selection. Although these three mechanisms have been considered in isolation by previous studies, this is the first study to provide data that are consistent with a synergistic interaction of all of them. Further understanding of the role of larval dispersal will require refined theoretical expectations for marine populations that are far from equilibrium, linked by complex patterns of dispersal, and in which a variety of contemporary and historical processes appear to be acting synergistically.
Literature Cited:


