UNIVERSITY OF CALIFORNIA, SAN DIEGO

The Effects of Size-Selective Harvesting on the Population Biology and Ecology of a Sex-Changing Limpet Species, *Lottia gigantea*.

A Dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Biology

by

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2008

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ACKNOWLEDGEMENTS

The completion of my dissertation would not have been possible without the help and inspiration from my friends, family, and academic colleagues. First, I would like to thank my advisor, Kaustuv Roy. His guidance and advice over the years has vastly improved the quality of my research and my understanding of ecology and evolutionary biology. Secondly, I need to thank Michael Hellberg, who effectively served as my "second" advisor. Without him and his lab group (Ron Eytan and Pat Arbour-Riley) at Louisiana State University, the last chapter of my dissertation would not have been possible. I would also like to thank my committee (past and present) for helpful discussions and advice; Josh Kohn, David Lindberg, Paul Dayton, David Holway, Ted Case, and Russ Lande. For field and lab assistance, I would like to thank Adam Calo. Three post-docs in the Roy lab gave me with encouragement and advice, Marcelo Rivadeneira, Gene Hunt, and Allen Collins.

My friends have provided me with an endless source of happiness over the last five years of my life here in San Diego. I would especially like to thank the superfriends and breakfast club members; Andrea Putnam, Boris Igic, Brielle Fischman, Steven Smriga, Tali Vardi, Kristen Marhaver, Jerry Boyd, Isaac Mehl, Rachel Borgatti, Stuart Bogatko, and Scott Bornheimer. I would not have kept my sanity without my weekly sports friends from the UCSD Beercats and Tuesday basketball (B.J. Haeck).

I would also like to thank my fellow EBE graduate student friends, Emma Goldberg, Sean Menke, Jennifer Sheridan, Tim Paape, Erin Wilson, and Meg Eckles. A special thank you goes to my labmate, Benajmin Pister.

My mom, dad, brother, sister and particularly my grandmother, Yetta (Baba) Schwartz, supported and encouraged my interest in science and natural history. Without them, none of this would have been possible. And last, but certainly not least, I would like to thank the 13,499 limpets that allowed me to measure them as a part of my dissertation.

Chapter 1 is a full reprint of the material as it appears in Fenberg, P. B., and K. Roy. 2008. Ecological and evolutionary consequences of size-selective harvesting: how much do we know? Molecular Ecology **17**:209-220. The dissertation author was the primary researcher and author of this publication and on all subsequent chapters.

This dissertation was made possible with support from the Environmental Protection Agency (EPA STAR fellowship), the Jeanne M. Messier Memorial Fund, and a California Sea Grant to Kaustuv Roy.

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Fenberg, P. B., and K. Roy. 2008. Ecological and evolutionary consequences of size-selective harvesting: how much do we know? Molecular Ecology **17**:209-220.

FIELDS OF STUDY

Ecology and Evolution, Conservation Biology

ABSTRACT OF THE DISSERTATION

The effects of size-selective harvesting on the population biology and ecology of a sex-changing limpet species, *Lottia gigantea*.

by

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Doctor of Philosophy in Biology

University of California, San Diego, 2008

Professor Kaustuv Roy, Chair

The selective removal of large individuals from a population (size-selective harvesting) is one of the most wide spread anthropogenic impacts of species in the marine environment. Size-selective harvesting can have numerous direct and indirect effects on the population biology, life history, and ecology of the target species and surrounding communities. This dissertation examines the cascading effects of size-selective harvesting on an ecologically important member of the rocky intertidal community of California, the Owl limpet, *Lottia gigantea*.

In the first chapter, I review what is known about the effects of size-selective harvesting across fish, marine invertebrates, and terrestrial vertebrates. In Chapter 2, I examine the effects of size-selective harvesting on the population biology and ecology of *L. gigantea* by comparing exploited field sites with well-protected field sites across a

large portion of the California mainland portion of its geographic range. I show that populations where *L. gigantea* is size-selectively harvested differ significantly in terms of size, abundance, biomass, and reproductive output compared to populations of this species that are protected from human harvesting. These differences also lead to changes in the structure of mid-intertidal communities, primarily due to the scaling relationship between size and space occupancy in *L. gigantea*.

In Chapter 3, I show that populations of *L. gigantea* are able to adjust a very important aspect of their life history (the timing of sex change) when subjected to size-selective harvesting. The relative size at which they change sex from male to female remains constant (~0.75) across populations subjected to varying degrees of harvesting pressure. This is the first study to measure all of the necessary life history parameters that are needed to test the theoretical predictions for an invariant relative size at sex change within a species.

In the fourth chapter, I use genetic methods to indirectly examine population connectivity of *L. gigantea* along the mainland coast of California. My results indicate that there are no significant barriers to gene flow between sampled field sites, suggesting that local populations of this species are demographically open.

CHAPTER 1

Ecological and evolutionary consequences of size-selective harvesting: how much do we know?

Molecular Ecology (2008) **17**, 209–220 doi: 10.1111/j.1365-294X.2007.03522.x

Ecological and evolutionary consequences of size-selective harvesting: how much do we know?

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Abstract

Size-selective harvesting, where the large individuals of a particular species are preferentially taken, is common in both marine and terrestrial habitats. Preferential removal of larger individuals of a species has been shown to have a negative effect on its demography, life history and ecology, and empirical studies are increasingly documenting such impacts. But determining whether the observed changes represent evolutionary response or phenotypic plasticity remains a challenge. In addition, the problem is not recognized in most management plans for fish and marine invertebrates that still mandate a minimum size restriction. We use examples from both aquatic and terrestrial habitats to illustrate some of the biological consequences of size-selective harvesting and discuss possible future directions of research as well as changes in management policy needed to mitigate its negative biological impacts.

Keywords: fishery, invertebrates, macroevolution, microevolution, size-selective harvesting, terrestrial vertebrates

Received 8 February 2007; revision received 11 July 2007; accepted 1 August 2007

Introduction

Body size is generally considered to be one of the most important traits of an organism because it correlates with many aspects of its biology, from life history to ecology (Peters 1983; Calder 1984). Size-selective harvesting, where large individuals of a particular species are preferentially taken, is a common practice in both terrestrial and marine habitats. Such harvesting practices are not only prevalent among people taking animals for food and other needs, but are also mandated by the management plans for many fish, invertebrate and game species. In fact, evidence for size-selective harvesting goes back to some of the earliest archaeological records of human settlement, dating back to at least the Middle Stone Age (Jerardino *et al.* 1992; Siegfried 1994; Mannino & Thomas 2002; Klein *et al.* 2004). It is not surprising that large individuals make up the bulk of the specimens in those archaeological deposits since they are the easiest to find and give the highest yield of protein per unit effort (Raab 1992). However, as subsistence and artisanal harvesting have given way to commercial exploitation and industrial fishing, an increasing number of species worldwide have been subjected to size-selective harvesting. There is growing evidence that decades of size-selective harvesting has led to the reduction in body sizes of many species and that such artificial selection against large body size affects not only the targeted species but also the surrounding community (see below). However, the effects of size-selective harvesting are multifaceted and often species and system specific. Thus, even though size-selective harvesting is increasingly being recognized as a cause for concern (Birkeland & Dayton 2005), so far the ecological and evolutionary consequences of this practice have been explored only for a limited number of species.

In this paper, we first provide an overview of the scope and nature of size-selective harvesting. In particular, we estimate the number and types of species that are known to have been affected by size-selective harvesting practices and look at how that information has changed over the last few decades. We then briefly review the effects of size-selective harvesting on life history, demography and ecology of the exploited species, and discuss the evolutionary consequences of such impacts. A potential complication here comes from the fact that in the case of some species, both terrestrial and aquatic, harvesting preferentially

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In the latter case, harvesting is usually both sex and size selective. While the bias towards aquatic species is likely to be real, our search also revealed that the scientific literature on size-selective harvesting is still relatively limited, and does not capture the true scope of the problem. The selective loss of larger individuals is an inevitable consequence of most commercial and recreational fisheries (Sluka & Sullivan 1998; Beard & Kampa 1999; Law 2000; Law 2001; Longhurst 2006), which suggests that the number of fish species estimated to be affected by size-selective harvesting even by our comprehensive search is too low. In addition, illegal poaching of terrestrial vertebrates also tends to be size selective (Milner-Gulland & Mace 1991; Milner-Gulland *et al*. 2003), but again our knowledge of the biological consequences of such harvesting is presently limited to only a handful of species. However, the number of exploited species for which we have some information in the peer-reviewed literature has been increasing over the last couple of decades (Fig. 1). The increase is evident for all the groups in our database, but is particularly strong for fish and invertebrates (Fig. 1). Furthermore, the trend is qualitatively the same whether we use the conservative or the comprehensive search (Fig. 1a).

The mechanisms used by commercial, recreational and artisanal fisheries and hunters to preferentially remove large individuals are almost as diverse as the number of species affected by such harvesting practices. For example, commercial fisheries tend to select larger fish through the use of different kinds of fishing gear such as trawls and gillnets (based on mesh size), longlines and trap nets (Bohnsack *et al*. 1989; Policansky 1993; Dahm 2000; Law 2000), while some recreational fisheries, such as spear fishing for groupers in the Florida Keys, involves searching for individuals over certain size thresholds (Sluka & Sullivan 1998). Actively searching for and selecting large individuals is also common when people harvest marine invertebrates such as abalone and limpets (Branch & Moreno 1994; Lindberg *et al*. 1998; Murray *et al*. 1999; Moreno 2001), or hunt large terrestrial vertebrates (Ginsberg & Milner-Gulland 1994). Interestingly enough, one of the largest sport hunting activities in the USA tends to preferentially remove the smaller rather than the larger individuals in a population. Duck hunters generally shoot individuals of lower overall condition (i.e. lower body mass) because they are more abundant at feeding decoys (Weatherhead & Ankney 1984). This is exactly the opposite of the trend seen for most other species where the largest and presumably most fit individuals are preferentially hunted. In fact, there are many documented examples of increased hunting mortality of lower condition ducks (Greenwood *et al*. 1986; Hepp *et al*. 1986; Reinecke & Shaiffer 1988; Dufour *et al*. 1993; Heitmeyer *et al*. 1993).

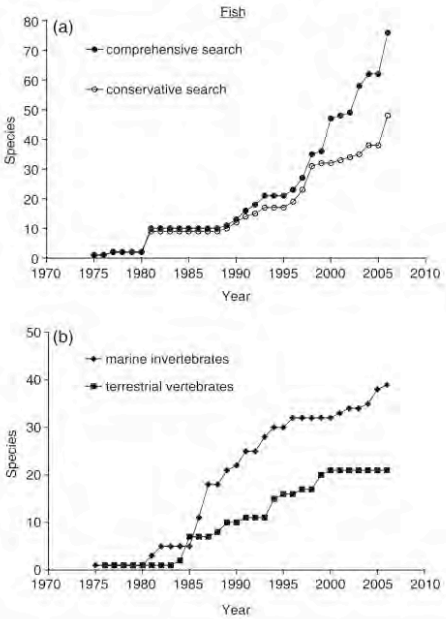

Fig. 1 (a) A plot of the cumulative number of fish species (marine and freshwater) reported to be affected by size-selective harvesting since 1975. The data points marked by closed circles represent the temporal accumulation of size-selectively harvested species using our comprehensive approach and those marked by open circles represent the results of our conservative approach (see text for details). Each species was added to the plot based on the earliest record (publication date) in our database of it being subjected to size-selective harvesting. (b) A plot of the cumulative number of marine invertebrate and terrestrial vertebrate species reported to be affected by size-selective harvesting since 1975. See text for details of the search protocols.

Consequences of size-selective harvesting

Size-selective harvesting can affect many aspects of the biology of an organism, from life history, demography, genetics and behaviour to the local abundance and biomass of populations. One of the biggest challenges to understanding the biological effects of size-selective harvesting is that many of the details tend to be taxon-specific. In some cases, it can also be difficult to distinguish between the effects of heavy but not necessarily selective exploitation and size-selective harvesting. As mentioned above, truncation of the large size classes and consequent changes in life history and demography are expected under both of these situations (Conover 2000; Heino & Godo 2002). For species

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where we have information about harvesting practices, it is relatively straightforward to determine whether a population has been subjected to size-selective exploitation. For example, the largest individuals of many intertidal invertebrate species are selectively harvested for food since they provide the most meat for the effort (Siegfried 1994). Similarly, trophy hunters almost always target large males (Ginsberg & Milner-Gulland 1994; Coltman *et al.* 2003; Milner *et al.* 2007). But in the case of commercially harvested marine fish, it can sometimes be difficult to determine how much of the observed changes in life history and demography are due to intense exploitation vs. increased fishing mortality of the largest size classes (i.e. size-selective harvesting). Most fisheries only catch individuals above a minimum size that is mandated by fisheries managers or determined by the gear (e.g. mesh size). Furthermore, historical data show that almost all fisheries start out by preferentially harvesting the large individuals (Jennings & Kaiser 1998; Jackson *et al.* 2001). Thus, in a strict sense, most commercial marine fisheries harvest individuals in a size-selective manner (also see Policansky 1993; Heino & Godo 2002). However, many marine species are currently under intense harvesting pressure and the minimum size mandated by management plans may be quite small relative to the maximum sizes that have been historically attained by individuals in exploited populations (see Jackson *et al.* 2001). In these species, most size classes rather than just the largest ones experience elevated mortality due to fishing, which can lead to changes in life history and demography. Clearly, defining what constitutes size-selective harvesting is somewhat arbitrary and it is important to recognize that most harvesting strategies can lead to increased adult mortality rates and a reduction in the number of large individuals present in a population. In the discussion below, where appropriate, we have attempted to separate the effects of size-selective harvesting per se from those due to intense exploitation (or overharvesting) but in some cases, it may be difficult to disentangle the two.

Changes in body size and mortality rate

The primary effect of size-selective harvesting and exploitation in general is an overall reduction in body size and an increased mortality rate of the harvested species. Body size declines attributed either partly or primarily to size-selective harvesting have now been documented in many species of marine and freshwater fish (e.g. Ricker 1981; Beard & Kampa 1999; Zwanenburg 2000; Harvey *et al.* 2006), marine invertebrates (e.g. Branch 1975; Siegfried 1994; Moreno 2001; Branch & Odendaal 2003; Roy *et al.* 2003) and some terrestrial vertebrates (e.g. Coltman *et al.* 2003). When size-selective harvesting targets a particular sex, reductions in body size are evident only for that sex. For example, hunting of bighorn trophy rams leads to a significant reduction in body size of males in the population (Coltman *et al.* 2003). In most cases, relatively little information exists about how long size-selective harvesting has been taking place or the rate of decline over time. Quantifying the rate of decline requires historical information about body sizes (Jackson *et al.* 2001) and although such information is potentially available for many species of marine invertebrates, so far only a few studies provide such analyses (e.g. Roy *et al.* 2003). In contrast, many studies of exploited fish species provide information on how sizes have declined over time (e.g. Handford *et al.* 1977; Ricker 1981; Zwanenburg 2000; Jackson *et al.* 2001; Harvey *et al.* 2006; Hsieh *et al.* 2006).

A decline in body size because of exploitation is also associated with changes in the mortality schedule of affected populations. There are two sources of mortality that natural populations have evolved with, intrinsic and extrinsic (Stearns 1992). Intrinsic sources of mortality are those that contribute to patterns of senescence and ageing, whereas extrinsic sources of mortality are associated with factors such as predation. Human harvesting acts to increase the extrinsic sources of mortality in affected populations to such an extent that for many species, it is the most common cause of adult mortality (Heino & Godo 2002; Festa-Bianchet 2003). Size-selective harvesting by humans is therefore a source of extrinsic mortality where the larger size classes experience higher harvest mortality than the smaller size classes. Although any increase in total mortality rate is expected to have an influence on life-history traits (Stearns 1992; Conover 2000), the response is likely to be more extreme when harvest mortality is size (age) specific (Stokes *et al.* 1993; Ginsberg & Milner-Gulland 1994; Conover 2000; Law 2000; Moreno 2001; Heino & Godo 2002; Milner *et al.* 2007). Thus, the net result of harvest-induced elevated mortality is an overall decline in the number of individuals surviving to older ages and larger sizes, which can lead to a multitude of cascading effects (see below).

Growth and survival of offspring

The quality of offspring is perhaps the most nonintuitive trait to be affected by size-selective harvesting, yet there is increasing evidence for such an effect in both marine and terrestrial species. The size and quality of larvae of some exploited marine fish has been shown to be positively correlated with maternal length and age (Berkeley *et al.* 2004). For example, older mothers of *Sebastes melanops* (black rockfish) provide larger oil globules for their larvae than younger and smaller females, which can enhance the growth rate and survival of the larvae (Berkeley *et al.* 2004). Similarly, older (larger) females can produce higher quality eggs, leading to enhanced survival of their larvae (Trippel 1995; Kjesbu *et al.* 1996; Vallin & Nissling 2000). Given these and other maternal effects, removal of the oldest and largest

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females has the potential to affect the size, growth and survival of larvae of a number of fish species (Vallin & Nissling 2000; Berkeley *et al.* 2004; Birkeland & Dayton 2005; Longhurst 2006). However, whether such maternal effects on larval quality are present in most fish species or whether they are clade specific remains unknown. Similarly, while maternal size has been shown to influence larval survival in some marine invertebrates such as bryozoans (Marshall & Keough 2004), it is unclear how common these characteristics are in invertebrate species that are exploited for human consumption.

Among terrestrial animals, some sex-selectively (and hence size-selectively) hunted ungulate populations show a reduction in offspring weight. For moose (*Alces alces*) and reindeer (*Rangifer tarandus*), reduced offspring weight may occur when females are forced to mate with young males after hunting reduces the number of older males (Saether *et al.* 2003; Holand *et al.* 2006; Milner *et al.* 2007). This reduction is not a direct result of lower male physiological health but an indirect result of delayed parturition dates for offspring sired by younger males (Milner *et al.* 2007). Female behavioural avoidance of less mature young males early in the season drives lower calf weight at birth later in the breeding season (Holand *et al.* 2006), which is a direct result of there being fewer large (older) males in the population because of sex-selective harvesting pressure. In the case of both grizzly bears (*Ursus arctos*) and African lions (*Panthera leo*), large and socially dominant males are also selectively hunted as trophies. This can result in an unfortunate side-effect of reduced survival among juveniles triggered by an increase in infanticidal behaviour by less dominant males (Swenson *et al.* 1997; Whitman *et al.* 2004; Loveridge *et al.* 2007). Once the dominant male has been removed from the population, the younger and peripheral males seek to increase their fitness by killing the offspring of the hunted dominant male and thus, reducing the interbirth period required to sire the next litter of cubs (Milner *et al.* 2007).

Reproductive investment

For long-lived species with low natural adult mortality rates, size-selective harvesting may cause shifts in life-history traits that are linked to adult survival, such as reproductive investment. The expected contribution to population growth of an individual (reproductive value) changes with age such that juveniles have low reproductive value (RV), mature adults have high RV, and in long-lived organisms, RV declines slowly with age (Kokko *et al.* 2001). It is therefore not surprising that size-selective harvesting of mature adults with the highest RV can lead to a decline in population growth rate. The most likely consequence of high adult mortality through harvest pressure is an increase in reproductive investment of young adults (Festa-Bianchet 2003). For hunted ungulate populations, subadult males are more likely to reproduce at a younger than normal age because of the reduced number of competitive older males during the rutting season (Milner *et al.* 2007). The effects of size-selective harvesting on the reproductive investment of invertebrate species are very poorly studied. But indirect evidence suggests that at least for some sex- and size-selective crab fisheries, where large males are preferentially taken, the surviving younger and smaller males attempt to reproduce more frequently than they normally would in the presence of larger and older males (Carver *et al.* 2005).

Growth and age (size) at maturity

Nearly all of the studies looking at the effects of size-selective harvesting on growth and age (size) at maturity have focused on fish (e.g. Handford *et al.* 1977; Spangler *et al.* 1977; Ricker 1981; Stokes *et al.* 1993; Law 2000; Heino & Godo 2002; Engelhard & Heino 2004; Baskett *et al.* 2005). Although a reduction in the age at maturity of harvested fish stocks is well documented, particularly for species with relatively late maturation times (Haug & Tjemsland 1986; Bowering & Brodie 1991; Rijnsdorp 1993; Trippel 1995; Rochet 1998; Law 2000; Grift *et al.* 2003), the underlying processes are not always clear (for an excellent review on this subject see Heino & Godo 2002). For example, fishing may indirectly select for either an increase or decrease in individual growth rates depending on any number of factors, including the size-selective nature of the harvest regime (Heino & Godo 2002). The size selectivity of the fishing gear and/or minimum size restrictions may preferentially remove faster growing individuals that 'recruit' to the fishery at a younger age, leaving individuals with a genetic tendency to grow more slowly. While some laboratory experiments have provided support for this hypothesis (Conover & Munch 2002), the extent to which selection for slow growth affects maturation times remains poorly known for a number of reasons. First, fishing, whether size selective or not, will reduce the stock abundance and decrease the total number of intraspecific competitors for food resources (Policansky 1993). This improvement in food access may result in accelerated juvenile growth and an overall younger age at maturation (Policansky 1993; Trippel 1995; Law 2000; Heino & Godo 2002; Engelhard & Heino 2004), potentially dampening any effects of selection for slower growth. Furthermore, harvesting-induced elevated mortality by itself (whether selective or not) is expected to lead to a very small number of individuals surviving to old ages and large sizes, leaving relatively young individuals to dominate the population. Individuals with a tendency to mature at an early age will contribute more of their genes to the next generation than individuals with a tendency to mature at older ages simply because of their probability of successfully reproducing before being harvested (Law

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2000; Heino & Godo 2002; Engelhard & Heino 2004). This concern that harvesting might cause genetic changes in growth or maturation times for exploited fish stocks was first put forth by Miller (1957) and later by Spangler *et al.* (1977), Handford *et al.* (1977) and Borisov (1978), but was not intensively examined until the early 1990s (Rijnsdorp 1993; Stokes *et al.* 1993). More recent studies have implicated genetic change as being partly responsible for observed changes in maturation times of some heavily exploited fish stocks (Grift *et al.* 2003; Barot *et al.* 2004; Olsen *et al.* 2004). But for most species, it remains unclear to what extent the observed changes in maturation time are due to phenotypic plasticity or evolutionary change (Grift *et al.* 2003). In addition, physical factors such as increased surface water temperatures caused by global warming can also contribute to accelerated juvenile growth rates and associated changes in life history (Thresher *et al.* 2007). Finally, because growth typically slows after maturation, a younger maturation time should result in a smaller size at age in the future (Heino & Godo 2002). Thus, regardless of the specific cause, a reduction in the age at sexual maturity of exploited fish stocks is likely to be followed by an overall reduction in yield (Law 2000; Conover & Munch 2002; Heino & Godo 2002; Ernande *et al.* 2004).

Whether these effects seen in fish also apply to other marine organisms remain unclear at present. As mentioned above, comparable studies of marine invertebrates are scarce, but studies of exploited marine limpets in South Africa and Costa Rica show surprisingly little impact of harvesting on growth rates and age at maturity (Ortega 1987; Branch & Odendaal 2003).

Fecundity and biomass

In fish and invertebrates, fecundity not only increases with size (Kido & Murray 2003; Birkeland & Dayton 2005) but in many species, relative fecundity (i.e. fecundity per gram of body weight) can be higher in older and larger individuals (Longhurst 2006). For example, reproductive output of a size-selectively harvested intertidal marine limpet *Cymbula oculus*, inside a marine protected area (MPA) was found to be 80-fold higher than that of exploited populations (Branch & Odendaal 2003). For this species, the biomass of protected populations was also substantially higher (30–90%) than exploited ones (Branch & Odendaal 2003), and similar differences exist in other species such as *Lottia gigantea*, an intertidal limpet from California that is also size-selectively harvested (P.B. Fenberg and K. Roy, unpublished). It is important to note that a reduction in biomass by itself does not necessarily indicate that harvesting is size selective; increased mortality due to harvesting in general is expected to reduce the standing biomass of exploited populations and it is now well documented that the current biomass of many exploited fish stocks represent a fraction of their historical levels (e.g. Jackson *et al.* 2001; Myers & Worm 2003). What separates the effects of size-selective harvesting from overexploitation is the observation that the former has the potential to change the fundamental scaling relationship between size and biomass predicted by macro ecological theory (Jennings & Blanchard 2004). When removal is size selective, the slope of the size–biomass relationship tends to change abruptly between size classes that are protected from fishing and those that are not, with the former slope being consistent with that predicted from energy-equivalence theory (Jennings & Blanchard 2004).

Changes in sex ratio

For some species size-selective harvesting can directly or indirectly lead to the preferential harvesting of one sex and thus has the potential to alter the breeding sex ratio. In the aquatic environment this is most commonly seen in sequentially hermaphroditic fish and invertebrates, where all individuals start out as one sex and then change to the other as they grow older and larger. Size-selective harvesting of these species thus preferentially removes the larger sex and can limit the reproductive potential of the population if it alters the sex ratios (Birkeland & Dayton 2005). As with age at maturity, life-history theory predicts that individuals should change sex at a younger age in response to high adult mortality and show changes in growth rate if sex change is under exogenous control (Warner 1975; Charnov 1979; Charnov 1981). If adult mortality rates are unnaturally high because of size-selective harvesting it is predicted that the age (size) at sex change will be reduced in order to compensate for the impacts on breeding sex ratio (Charnov 1981; Armsworth 2001; Platten *et al.* 2002). Such trends are evident in some size-selectively harvested fish (Cowen 1990; Buxton 1993; Platten *et al.* 2002; Hawkins & Roberts 2004) and invertebrate species, particularly shrimp (Charnov 1981; Hannah & Jones 1991). On the other hand, this compensatory response may not occur if harvesting pressure is intense enough to not allow adequate time for sex change (Coleman *et al.* 1996; Hawkins & Roberts 2004). Additionally, if size at sex change is fixed, as it appears to be for some species (Branch & Odendaal 2003; Munday *et al.* 2006), then early age (size) at sex change is not likely under harvesting pressure. In these cases, the loss of larger individuals leads to drastic changes in the population sex ratio (Branch & Odendaal 2003).

Even for non-hermaphroditic aquatic species, size-selective harvesting can disproportionately affect one sex. Many crab and lobster fisheries preferentially harvest (directly or indirectly) the larger males in the population, resulting in female-biased sex ratios (Paul & Adams 1984; Wenner 1989; Smith & Jamieson 1991; Castilla *et al.* 1994; Sato *et al.* 2005; Sato & Goshima 2006). A skewed sex ratio can limit

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the reproductive potential of fished populations via sperm limitation or delayed mating (Sato & Goshima 2006). However, field observations are usually difficult and only indirect evidence exists for the effect of male-focused crustacean fisheries on reproductive success (Smith & Jamieson 1991; Carver *et al.* 2005; Sato *et al.* 2005).

In some freshwater eels, sex is environmentally determined with males differentiating at a younger age and smaller size than females, and extreme male biases found in harvested eel populations have been partly attributed to the size-selective nature of the fishery (McCleave & Jellyman 2004; Beentjes *et al.* 2006). Population sex ratios can be more directly impacted when harvesting is both size and sex selective. For terrestrial species, a female-biased sex ratio is perhaps the most common direct effect of sex-selective hunting (Ginsberg & Milner-Gulland 1994; Milner *et al.* 2007). However, at least for polygynous species, a skewed sex ratio is commonly assumed to have little negative effect on population growth since one male can potentially inseminate many females (Caughley 1977; Ginsberg & Milner-Gulland 1994). In fact, recruitment rates for some ungulate populations are resilient to unnaturally skewed sex ratios (Milner *et al.* 2007) and somewhat ironically, male-selective hunting may have actually contributed to an increase in population growth rate and the eventual overabundance of deer populations across much of Europe and North America (along with other factors such as reduced natural predation and increased food availability; Cote *et al.* 2004). But of course, there is a limit to the bias in sex ratio on the long-term viability of a population. Population crashes attributed to reduced fecundity of sex-selectively hunted ungulate populations have been documented for species such as the saiga antelope (*Saiga tatarica tatarica*) (Milner-Gulland *et al.* 2003; Milner *et al.* 2007).

Ecological effects

There is increasing recognition that exploitation has ecological consequences and can lead to large changes in community composition and the functioning of ecosystems. Much of this work has focused on the effects of overexploitation of fish species (Tegner & Dayton 1999), and it can be difficult to separate the ecological effects of overexploitation from that of size-selective harvesting. Moreover, the ecological consequences of harvesting depend, at least partly, on the functional role and competitive dominance of the target species (Kaiser & Jennings 2001). Thus, the effects are system specific and sometimes quite complex. In Chile, size-selective harvesting reduces the size and abundance of the large limpet *Fissurella picta*, resulting in an increased abundance of its macro-algal food source (Moreno *et al.* 1984; Godoy & Moreno 1989). In areas where *F. picta* is harvested (usually size-selectively), a co-occurring but nonharvested smaller limpet *Siphonaria lessoni* with a similar diet grows faster and reaches a bigger size in the absence of large individuals of the competitively dominant *F. picta* (Moreno *et al.* 1984; Godoy & Moreno 1989). Similarly, increases in the abundance of a sea urchin *Arbacia lixula*, has been attributed to size-selective harvesting of its competitor *Paracentrotus lividus* (Guidetti *et al.* 2004). In general, selective harvesting of some species can increase the growth rate, size and abundance of other nonharvested species because of release from competitive pressure (Godoy & Moreno 1989; Lindberg *et al.* 1998; Guidetti *et al.* 2004). Similar indirect effects should also be common where larger individuals of the target species are highly territorial. For example, a number of intertidal limpet species territorially defend their algal grazing area by 'bulldozing' any intruders such as barnacles, mussels and even other conspecifics (Stimson 1970; Branch *et al.* 1992; Shanks 2002). Selective loss of large individuals of these species can result in community level shifts in space occupancy (Griffiths & Branch 1997; Lindberg *et al.* 1998), which may be difficult to reverse even after harvesting is relaxed. As far as indirect effects are concerned, one of the most extreme examples come from the Canary Islands where size-selective harvesting of intertidal limpets may have partially contributed to the extinction of the oystercatcher *Haematopus meadewaldoi* (Hockey 1987; Branch & Moreno 1994).

Size-selective harvesting can also have an impact on behavioural ecology. One of the better examples of this comes from African elephants (*Loxodonta africana*) where hunters preferentially kill the largest and oldest elephants from a population for the international ivory trade (Milner-Gulland & Mace 1991). In non-hunted populations, male elephants typically enter a state of heightened sexual activity and aggressive behaviour known as musth between 25 and 30 years of age. At this age, they have become large and competitive enough to win encounters with other males (Poole 1987; Poole 1989; Slotow *et al.* 2000). However, hunted populations consist of inexperienced young males with smaller tusks. The lack of an older male hierarchy in these populations can cause the young males to enter musth at ages as young as 18 years old (Slotow *et al.* 2000). An unexpected side-effect of their inexperience and heightened aggression associated with entering musth at an early age is an increased incidence of young males attacking and killing individuals of rhinoceros (*Diceros bicornis* and *Ceratotherium simum*, Slotow *et al.* 2000; Slotow *et al.* 2001). This behaviour is uncommon in populations with normal age structures, and conservation managers have been able to solve the problem by introducing older males to some of the affected populations (Slotow *et al.* 2001).

Microevolution or phenotypic plasticity?

Size-selective harvesting clearly causes large and observable changes in life history and ecology of exploited species,

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but whether these are evolutionary responses (i.e. have a genetic basis) or whether they represent phenotypic plasticity remain unclear (Law 2000). Laboratory experiments have shown that in some fish size-selective harvesting can select for genotypes with slower or faster growth rates depending on whether large or small individuals are selectively removed (Conover & Munch 2002). Thus, in principle, size-selective harvesting can lead to rapid evolutionary response and analyses of some wild populations have found evidence for such a response (Coltman *et al.* 2003; Grift *et al.* 2003; Olsen *et al.* 2004). Similarly, the failure of traits such as size at maturity to return to pre-exploitation levels when fishing is stopped is consistent with an evolutionary response (Conover & Munch 2002). On the other hand, the size of many species tends to increase once they are protected from exploitation (Halpern & Warner 2002; Branch & Odendaal 2003; Gell & Roberts 2003; Roy *et al.* 2003; Hawkins & Roberts 2004), suggesting that some of these changes reflect plasticity. In general, for the vast majority of exploited species, the information required to differentiate between evolutionary change and phenotypic plasticity is currently not available (Conover 2000). In fact, even though size-selective harvesting is widespread, we know little about the magnitude of selection differentials due to such exploitation (Stokes & Law 2000; Law 2001). Finally, for most exploited stocks, we have little quantitative data on how the exploitation pressure has varied over time. Some authors view changes in many marine fish stocks as a relatively recent phenomenon (i.e. latter half of the 20th century; Hutchings & Baum 2005), while others argue that such declines extend back a couple of centuries or longer (Jackson *et al.* 2001). Both views are probably correct given that humans have been exploiting some species of fish and invertebrates for thousands of years (Jerardino *et al.* 1992; Jackson *et al.* 2001; Klein *et al.* 2004) while other fisheries are much newer. From an evolutionary standpoint, the lack of such information makes it difficult to estimate how many generations have been subjected to selective harvesting. For taxa with a short or moderate lifespan, tens or maybe even hundreds of generations have already been subjected to size-selective harvesting, enough time for evolutionary responses (Conover & Munch 2002). On the other hand, many species of marine fish and invertebrates live for multiple decades and for these long-lived taxa, it may be too soon to see evolutionary changes even though they are likely in the long run (Conover 2000).

Macroevolutionary consequences of size-selective harvesting

Size-selective harvesting is pervasive and there is no indication that the situation is going to change in the near future. Thus, it is reasonable to expect that such selection pressure would lead to reduction in body sizes of many species, especially given the high heritability of this trait and its close relation to fitness (Law 2001). As discussed above, such an evolutionary response has already been documented in some species. In other cases it is likely to be present but is yet to be detected. While virtually all of the discussion about evolutionary response to size-selective harvesting has focused on microevolution, size declines due to such exploitation also have macroevolutionary implications. In fact, body size is often thought to provide a direct link between microevolution and macroevolution (Jablonski 1996). The patterns and mechanisms of body size evolution at the species level have been studied in considerable detail and empirical data show that in many clades average body size tends to increase over time as the clade diversifies, a trend commonly known as Cope's Rule (Jablonski 1996; Alroy 1998; Hunt & Roy 2006). At present, there are two general explanations for this trend. Stanley (1973) suggested that most major clades tend to originate at small sizes, and as they diversify, they add both large and small species. However, because of a hard lower bound for body size, the ultimate result of such passive diffusion is an increase in both mean and variance in body size over time (Stanley 1973; Gould 1988; Jablonski 1996). Alternatively, Cope's Rule can result from directional selection towards larger body sizes (Brown & Maurer 1986; Jablonski 1996; Hunt & Roy 2006). In either case, the natural tendency of many clades is to add larger-bodied species over time. Human exploitation has the potential to disrupt this evolutionary trend by truncating the larger end of the size distributions as body sizes of many species get smaller because of size-selective harvesting, and many large-bodied species face extinction because of other anthropogenic impacts (Gaston & Blackburn 1995; McKinney 1997). Under such a scenario, new species in the future are likely to be small since they will be derived from small ancestors and body size is highly heritable even at the lineage level (Smith *et al.* 2004). In addition, size-selective harvesting and other human activities counteract the selective advantages of large body size and would thus reinforce the bias against large-bodied species.

Conclusions

Harvesting of natural resources by humans is selective by nature (Law 2001; Longhurst 2006) and archaeological data show that such exploitation has been going on since the dawn of civilization (Klein *et al.* 2004). Size-selective harvesting is just one example of such selective exploitation, but because body size correlates with so many different attributes of an organism, such exploitation has far-reaching ecological and evolutionary consequences. Arguably, the difference between the reductions in body size seen in archaeological kitchen middens (Jerardino *et al.* 1992) and those due to fishing over the last couple of centuries is essentially one of scale. Today, size-selective harvesting

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affects many species and exploitation pressure is higher than ever before (Pauly *et al*. 1998; Baum *et al*. 2003; Myers & Worm 2003; Hutchings & Baum 2005). Yet we know very little about the evolutionary and ecological consequences of such exploitation. The majority of the information regarding changes in species life histories in response to size-selective harvesting have come from a handful of commercially important fish, largely from waters off developed countries (Hutchings & Baum 2005). The combination of taxonomic, geographical (little information exists for highly diverse tropical areas) and habitat-related bias (Hutchings & Baum 2005) makes it impossible to reach any general conclusions regarding the effects of size-selective harvesting. The situation is particularly bad for invertebrates where many species are harvested but not only do we lack information about their life history and ecology, but also reliable data on patterns of exploitation. Large databases of catch-rates and other information that permit stock assessments and analyses of population trajectories of many commercially important fishes (e.g. Baum *et al*. 2003; Myers & Worm 2003) are virtually unknown for most marine invertebrates.

Despite the paucity of specific information for many species, it is quite clear that size-selective harvesting is having a negative effect on the population biology of many species of vertebrates and invertebrates. Yet the problem is not recognized in most management plans for fish and marine invertebrates that still mandate a minimum size restriction (Conover & Munch 2002). In addition, illegal size-selective harvesting of intertidal invertebrates is a growing but under-appreciated problem in many parts of the world and even where regulations exist, they are rarely enforced (Branch & Odendaal 2003; Roy *et al*. 2003). Despite all this, the increases in size and biomass of exploited species within MPAs suggest that for many species, it may not be too late to reverse the negative ecological and evolutionary consequences of size-selective harvesting (Halpern & Warner 2002; Roy *et al*. 2003). However, achieving that would require us to stop preferentially removing the larger and older individuals in a population and design harvesting strategies that would preserve the size-frequency distributions that characterize the unexploited state of a species. Suggestions regarding such strategies are already available in the literature (e.g. Conover & Munch 2002; Jennings & Blanchard 2004; Birkeland & Dayton 2005; Hutchings & Baum 2005). More generally, mitigating the effects of size-selective harvesting would require us to shift from management strategies that are designed to maximize yield (Longhurst 2006) to those that can preserve the natural variations that characterize species and ecosystems.

Acknowledgements

We thank Thomas Smith and Louis Bernatchez for the invitation to participate in this summit and G. H. Engelhard and an anonymous reviewer for insightful comments on a previous version of this manuscript. This work was supported by a grant from NOAA California SeaGrant (to K.R.) and an EPA STAR Fellowship (to P.B.F.).

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Chapter 1 is a full reprint of the material as it appears in Fenberg, P. B., and K. Roy. 2008. Ecological and evolutionary consequences of size-selective harvesting: how much do we know? Molecular Ecology **17**:209-220. The dissertation author was the primary researcher and author of this publication.

CHAPTER 2

The effects of size-selective harvesting on the population biology and ecology of the territorial limpet, *Lottia gigantea*.

Introduction

Body size is arguably one of the most important traits of an organism because it correlates with many different aspects of its biology, from life history to ecology (Peters 1983; Calder 1984; Hildrew et al. 2007). Unfortunately, decades of human harvesting of wild animal populations for food and other needs have significantly altered the size-structures of many species, particularly fish and coastal invertebrates (Fenberg & Roy 2008). Humans typically harvest the larger size-classes of a species (size-selective harvesting), and the affected populations tend to be dominated by smaller (younger) individuals. Size-selective harvesting can have cascading effects not only on the life history and demography of the target species, but also on the structure and functioning of surrounding ecological communities. For example, the larger individuals are often the best competitors for resources, and their loss can potentially result in a relaxation of competitive pressures, both within a population and the ecological community as a whole (Moreno et al. 1984; Godoy & Moreno 1989; Lindberg et al. 1998; Slotow et al. 2000; Slotow et al. 2001; Guidetti et al. 2004). A number of studies have examined the ecological consequences of harvesting in fish communities, but because most of the species involved are commercially harvested, it can be difficult to attribute many of those effects to size-selective harvesting per se rather than over-exploitation (Fenberg & Roy 2008). The demographic and ecological effects of size-selective harvesting are better documented for some species of rocky intertidal and shallow subtidal invertebrates, particularly those from Chile and South Africa (Moreno et al. 1984; Hockey 1987; Godoy

& Moreno 1989; Siegfried 1994; Griffiths & Branch 1997; Branch & Odendaal 2003; Guidetti et al. 2004). But along the highly populated and urbanized coastline of western North America, where many intertidal invertebrates are routinely harvested for food and other needs (Murray et al. 1999), little is known about the demographic and ecological consequences of such harvesting (but see Lindberg et al. 1998; Roy et al. 2003).

Unlike many harvested fish species, rocky intertidal invertebrates have relatively low mobility once they have settled on the substrate as juveniles. This allows us to measure basic population level parameters such as size-structure and abundance in the field with relative ease. And for some species, such as territorial limpets (Stimson 1970; Branch et al. 1992; Branch & Moreno 1994), we can indirectly observe certain competitive interactions between other species that can help us understand some of the ecological consequences of size-selective harvesting.

Here, we present the results of a comprehensive study on the consequences of size-selective harvesting on a competitively dominant limpet species in western North America, the Owl limpet, *Lottia gigantea*. Our results highlight the efficacy and importance of well-protected areas (e.g. marine protected areas) while increasing our knowledge of the basic yet understudied ecological effects of size-selective harvesting in rocky intertidal communities.

Lottia gigantea is the largest Patellid gastropod species in North America, ranging from northern California (~39ºN) to southern Baja California (~26ºN). It is a long-lived

species with individuals capable of reaching ages of nearly 20 years (Fenberg and Roy in prep). *L. gigantea* has been exploited as a food source for over 13,000 years (Lindberg et al. 1998). However, exploitation patterns are more wide ranging and intense today compared with subsistence collecting in the past (Pombo & Escofet 1996; Lindberg et al. 1998). In fact, it has been suggested that most mainland populations of this species are currently affected by size-selective harvesting pressure (Pombo & Escofet 1996), which ultimately reduces the mean and maximum body size of affected populations (Pombo & Escofet 1996; Roy et al. 2003; Sagarin et al. 2007). Historical data corroborate such observations, showing that the largest size classes have significantly declined over the last 100 years in southern California (Roy et al. 2003). Much of the harvesting along the California coast is done illegally; so reliable statistics on catch rates are virtually non-existent (California Fish and Game personal communication). However, a small number of *L. gigantea* populations along the California mainland are well protected from harvesting pressure, either from restricted access or through effectively managed MPA's (Roy et al. 2003; Sagarin et al. 2007). Along with historical data, these populations provide us with a valuable comparative framework in which to examine the demographic and ecological effects of size-selective harvesting.

Lottia gigantea is a protandric hermaphrodite, with all individuals starting their reproductive lives as male and changing sex to become female as they grow older and larger (Wright & Lindberg 1982; Wright 1988, 1989). Females are also highly territorial of their feeding space, which consists of a thin layer of microalgae on the rocky substrate (Stimson 1970; Wright 1988, 1989; Shanks 2002). When partially or fully submerged,

these individuals actively patrol their grazing territory (or 'garden') by 'bulldozing off' non-territorial males, juveniles and interspecific intruders (i.e. other mobile gastropods), including any settling larvae with the anterior portion of their shells (Stimson 1970; Shanks 2002). They also prevent the encroachment of sessile species such as mussels, anemones, macroalgae and barnacles (Stimson 1970). Males and interspecifics generally reside outside of a territory or within areas difficult for the larger females to reach (i.e. cracks in the substrate). However, *L. gigantea* males have been known to raid female territories and eat as much microalgae as they can before being 'chased' away by the female (Shanks 2002).

By preferentially removing large individuals, size-selective harvesting acts to remove the *L. gigantea* individuals that occupy the most space, leaving populations to be dominated by less space competitive (i.e. smaller) individuals without the need or capacity to occupy large territories. Previous studies have experimentally removed large territory holders, showing that other mobile (including other *L. gigantea* individuals) and sessile species establish themselves on previously occupied spaces (Stimson 1970; Lindberg et al. 1998). Because *L. gigantea* individuals are generally not capable of removing other highly space competitive interspecific individuals or colonies once they have established themselves (e.g. mussels and barnacles; Stimson 1970), size-selective harvesting of *L. gigantea* populations has the potential to alter the rocky intertidal community structure and functioning by affecting space occupancy. However, no study to date has quantified the extent of this ecological effect by comparing protected and exploited *L. gigantea* populations and communities.

To understand the effects of size-selective harvesting on *L. gigantea* populations, we compared populations from protected and exploited sites to quantify changes in (1) size-structures, (2) population abundance, and (3) overall biomass and reproductive output. In addition, we explored whether the harvesting of this species has any cascading effects on the composition of the surrounding rocky intertidal community. Finally, we use a compilation of historical data on body sizes of this species to make inferences about how size-selective harvesting has affected site-specific patterns of size decline and space occupation by *Lottia gigantea* on a century scale.

Materials and Methods

Study sites

We measured the abundance and size-structure of *Lottia gigantea* at 10 field sites in California (Fig. 2-1 and Table 2-1). We classified each site into one of two general categories - exploited and well-protected- based on their level of harvesting pressure. Our protocol for placing a field site within the exploited category was based on observations of the extent of historical decline in body size (in the case of the Palos Verdes populations; see below) and direct observations of poaching events (Fig. 2-2 SIO; Don Canestro personal communication at Ken Norris UC reserve). Protected populations were classified as such based on whether a substantial number of individuals within the population reached sizes close to the historically recorded maximum for this species (i.e. 85-100 mm in length). Where appropriate, we also used the vulnerability protocol outlined in Sagarin et al. 2007, to classify field sites in each category. By our measures,

three out of the ten field sites within this geographic area were considered to be well-protected from harvesting; Cabrillo National Monument, Vandenberg Air Force Base, and Pebble Beach Golf Course. These protected sites are approximately equally spaced across our entire study area (separated by 2º of successive latitude from 32.66ºN - 36.56ºN; Fig. 2-1). This equal spacing allows us to better detect any natural latitudinal trends in size-structure and abundance that may otherwise be obscured if the protected sites were geographically clumped. The remaining seven sites are classified as exploited (Cortez, Scripps UC reserve, 1000 steps, three sites on the Palos Verdes peninsula and Ken Norris UC reserve).

Sampling methods

We sampled our field sites during low tides from 2003 to 2006 by placing belt transects of meter square quadrats in suitable *L. gigantea* habitat. The length of each individual within a quadrat was measured to the nearest millimeter using calipers and marked with chalk to avoid sampling duplication. We avoided sampling in areas with highly rugose surfaces in order to eliminate habitat effects on the size-structure in our data (Kido & Murray 2003). However, previous researchers have suggested that the occurrence of large *L. gigantea* individuals is not well predicted by substrate differences between sites (Sagarin et al. 2007). Because individuals of this species are non-randomly distributed across the rocky intertidal, we were careful not to sample quadrats that fell on areas unoccupied by *L. gigantea*. Therefore, the lowest number of individuals within a quadrat in our dataset is one.

We took special care to find and measure all individuals over 10 mm in order to get an unbiased estimate of size-distribution and abundance at each field site. Individuals below 10 mm cannot be distinguished from other limpet species found within the same habitat. After each quadrat was searched, we took a high-resolution digital photograph of the quadrat for analyses in the lab (see below). We sampled most populations over the course of two successive days. In total, we measured 7,661 individuals from the 10 field sites for this study (protected n=1,844, exploited n=5,817).

We collected 173 individuals ranging in size from 18 to 70 mm from four sites and weighed each individual to the nearest gram (wet weight plus shell) to obtain a length-mass relationship for biomass estimates. We collected these individuals at the height of the spawning season in southern California (December – February), and dissected out the gonads of each individual and weighed them to the nearest gram using a protocol outlined in Kido and Murray (2003) to create a length-gonad mass relationship. We used a second order polynomial regression of length by mass (r^2= 0.97, $p < 0.0001$) and length by gonad mass (r^2= 0.60, $P < 0.0001$) to estimate the average biomass and reproductive output per square-meter for each sampled population. We only included individuals over 25 mm (~2 years old) for estimates of reproductive output because the smaller size classes tend to be immature (Fenberg unpublished data).

Our goal was to highlight the differences between protected and exploited sites and not necessarily to compare pairwise differences across all sites. Therefore, to test for differences in body size, abundance, biomass and gonad output, we compared the mean

values of these measurements between protected (n=3) and exploited sites (n=7) using non-parametric tests. We also created bivariate plots of these measurements to visualize their differences.

Territory / home range scaling relationships

We measured the lengths of 104 individuals and the areas of their respective territories / grazing areas from protected and exploited sites. Individual territories can often be identified by the distinctive radula marks left in the algal film by the territory holder, which often resides within a home scar at the edge of its territory at low tide (Fig. 2-3). The area of each territory (mm^2) was measured using a digital photograph of the territory and image J software (version 1.34s). We measured the territory sizes only for individuals living on flat surfaces due to the difficulty of measuring area for highly rugose surfaces. We chose two well-protected sites (Cabrillo National Monument and Vandenberg Air Force Base) and two exploited sites (Ken Norris Rancho Marino UC Natural Reserve and Pt. Fermin) for our analyses. It was important to include sites with varying degrees of harvesting pressure because it is necessary to measure the territory sizes for individuals across a large range of size classes. For example, large size classes do not overlap between protected and exploited sites, and intermediate size classes at protected sites are less likely to hold territories compared to individuals of the same size at exploited sites. We performed linear regression models for exploited and protected populations separately and together to look for differences in the scaling relationships.

Mid-intertidal space availability

We estimated the amount of space available for the feeding and growth of *L. gigantea* individuals in the mid-intertidal using 80 quadrats from protected (n=40) and exploited sites (n=40). Using our digital photographs (see above) and Image J software, we placed 50 equally spaced points within each quadrat and recorded whether the points fell on grazing space occupied by *L. gigantea* individuals or on another organism (e.g. barnacles, chitons, mussels, macroalgae, or other limpet species). A point was defined as grazing space if it fell on a rock surface with *L. gigantea* radular marks. When radular marks were not visible, if a point fell immediately adjacent to *L. gigantea* individuals, it was assumed to be part of a grazing area or territory. If a point fell on an *L. gigantea* individual, we also counted it as grazing space. We divided the number of grazing space points by the total number of points to get a percentage of grazing space for each quadrat. We were careful to only include quadrats that were placed in the mid-intertidal because the higher intertidal (where *L. gigantea* also occur) is commonly characterized by bare space due to desiccation stress and in that habitat, competition for space appears to be less intense. In addition, there are many other small limpet species that occur in the higher intertidal and it can be difficult to separate grazing areas specific to *L. gigantea*. We used a t-test to determine if protected sites have significantly more available grazing space compared to exploited sites.

Historical data

We measured the body size (longest shell length), geographic locality, and year collected for every *L. gigantea* individual within the collections at each of the five major natural history museums in California (The San Diego, Los Angeles County and Santa Barbara Museums of Natural History, University of California Museum of Paleontology, and the California Academy of Sciences) and the National Museum of Natural History, resulting in a database of 2,552 occurrences dating back to 1869 (mainland and island localities). For our analyses of historical size decline, we only used individuals collected from the Palos Verdes peninsula (~33.7ºN) in Los Angeles County where the mode of the mainland distribution of occurrences come from (Fig. 2-4) and where much of the historical and modern harvesting of *L. gigantea* in California has occurred.

We binned the occurrence data into ten-year intervals starting from 1904 and recorded the year and maximum shell size collected within each bin. We used our field data (ending in 2005) from three sites on the peninsula for the last data point (see below). We performed linear regression analyses to test the hypothesis that maximum body size has declined over the last 100 years on the Palos Verdes peninsula. In addition, we used non-parametric statistics to examine whether the distribution of museum *L. gigantea* shells collected prior to 1960 on the peninsula are larger than the modern distribution of individuals measured at this same location.

Results

Size structure

Previous studies have shown that the best predictor of the size structure of modern *L. gigantea* populations is the degree of protection they receive from harvesting (Roy et al. 2003; Sagarin et al. 2007). Our results corroborate these findings, showing that mean and maximum sizes of *L. gigantea* at our well-protected sites are significantly larger than those from exploited sites (K-S test on $\log_{10}$ mean sizes $p = 0.016$; K-S test on $\log_{10}$ maximum sizes $p = 0.029$). In addition, there are no latitudinal trends in either mean or maximum body size across sampled field sites (mean length $p = 0.26$; max length $p = 0.16$); the two sites at the latitudinal extremes of our study area, the protected sites Cabrillo NM (32.66ºN) and Pebble Beach (36.56ºN), have the same mean size (CNM: 50.0 mm; PBL: 50.4 mm) and similar maximum sizes (CNM: 91 mm; PBL: 97 mm).

Abundance, biomass and reproductive output

Exploited sites have significantly higher mean abundances compared with protected sites (K-S test on $\log_{10}$ mean abundance $p= 0.016$). However, this disparity does not correspond to significantly higher biomass ($\log_{10}$ mean grams per m^2; K-S test $P = 0.016$) or gonadal output (log mean gonad grams per m^2; K-S test $p = 0.016$). On average, protected populations contain 224 grams of *L. gigantea* per m^2 (SD 27.3, n=3), whereas exploited sites contain 119 grams of *L. gigantea* per m^2 (SD 57.5, n=7). This difference in biomass corresponds to an average difference of 3.61 grams of gonad mass per m^2 between exploited (2.19g per m^2; SD 1.25, n=7) and protected sites (5.80g per m^2; SD .874, n=3). Bivariate plots in Figure 2-5 show these differences between mean

abundance and mean size/biomass at protected and exploited sites. Note that the protected sites cluster together in each plot while the exploited sites are more scattered and variable. No latitudinal trends exist with respect to abundance (p=0.10) or biomass (p=0.53) across sites within our study area.

Territory / home range scaling relationships

Figure 2-6 shows the strong positive relationship between body size and territory (grazing area) size across all individuals (N = 104; log area = -0.546+2.99*log length; r^2 =.756, P<0.0001). When analyzed separately, there is no significant difference in this scaling relationship between protected and exploited sites even though the largest size classes and hence, largest space occupiers, do not overlap (Fig. 2-6).

Mid-intertidal space availability

There is significantly less grazing area available for *L. gigantea* individuals in the mid-intertidal at exploited sites compared to the protected sites (Fig. 2-7; t-test; DF 78 P<.0001). On average, 62.6% of the space within a 1 m^2 quadrat placed in the mid-intertidal habitat at protected sites is part of a *L. gigantea* territory or grazing area. In contrast, in the same habitat an average of only 40.4% of the quadrat space is used by individuals of *L. gigantea* at exploited sites. Besides *L. gigantea*, macroalgae (18%), mussels (7.4%), chitons (5.4%), barnacles (3.7%), and other limpet species (2.9%) make up the community composition of mid-intertidal quadrats at well-protected sites. At the

exploited sites however, the reduction in the space occupied by *L. gigantea* is made up by other species, such as macroalgae (25.8%), mussels (17.4%), barnacles (14.9%), other limpet species (1.1%) and chitons (.4%).

Historical changes in body size

Nearly a quarter (22.1%) of the museum specimens of *L. gigantea* within our study region occur on the Palos Verdes peninsula (~33.7ºN) in Los Angeles County. In fact, this peninsula has historically been the most widely sampled area anywhere within the range of *L. gigantea* (Fig. 2-4). With 305 occurrences dating back to 1904, Palos Verdes is an ideal location to examine historical changes in body size.

A significant negative correlation of maximum size by year (Fig. 2-8; $r^2 = 0.45$; $p < 0.05$) shows that maximum size of *L. gigantea* has declined by about 25mm over the last 100 years on the Palos Verdes peninsula (90.3 mm in 1904 – 66 mm in 2005). This trend is further exemplified by a comparison of size-frequency distributions of all of the museum specimens collected before 1960 with our field data for the peninsula (K-S test $p<0.0001$; Fig. 2-9). We could not find any individual that came close to the historical sizes attained by *L. gigantea* at this geographic locality even though we measured an order of magnitude more individuals in the field (n=2042) than what occurs in the museum data (n=208; pre-1960). This a conservative estimate of size decline because it is unlikely that the historically largest individual living on the peninsula was collected and archived in museum collections. In fact, a size decline of more than 50 mm is

possible given that the historically largest *L. gigantea* individual *ever* collected for a museum lived just north of the peninsula at 34.38ºN (116.5mm collected around 1900). Similar analyses of size decline at other localities in California are more limited because of smaller sample sizes. However, a conservative estimate of historical size decline at Bird Rock in La Jolla, CA (32.83ºN) is 13 mm (historical maximum from ~1940 = 89 mm; modern maximum = 76 mm).

Discussion

The modern effects of size-selective harvesting (abundance, biomass and reproductive output)

Our comparative analysis of modern protected and exploited populations of *L. gigantea* show that size-selective harvesting of this species is not only affecting a number of different aspects of the population biology of this species, but is also changing the composition of the surrounding ecological community. As stated earlier, the difference in mean and maximum body size between exploited and protected sites is a direct result of size-selective harvesting pressure, but the cascading effects of reduced body size on other aspects of the population biology of *L. gigantea* and surrounding communities is more complicated (see Fig. 2-10).

Harvesting by humans should reduce the abundance of a species, but in the case of *L. gigantea*, harvested populations tend to have higher overall abundance. The primary

reason for this appears to be the territorial nature of larger individuals of this species and the scaling relationship between territory size/grazing area and body size (Fig. 2-6). The relationship shows that larger individuals from protected sites occupy proportionally more space compared smaller individuals from exploited sites. Since large territorial individuals actively exclude other conspecifics (generally non-territorial males) from their feeding space, a population consisting of large individuals with concomitantly large territories (i.e. well-protected sites) should have lower overall abundances per unit area compared with a population made up of smaller (and hence less space competitive) individuals (i.e. exploited sites). However, it is important to note that not all individuals in a population of *L. gigantea* are territorial. Small non-territorial males tend to haphazardly roam within a territory before being chased away by a female (Shanks 2002) personal communication Stephanie Schroeder). Previous field studies have even shown that territorial individuals will dislodge smaller intraspecific invaders from the substrate (Stimson 1970). Because of this, many individuals, most notably immature juveniles (~25 mm and below and 2 years old and younger), reside and graze in areas that are difficult for territorial females to reach, either within cracks in the substrate or on the tops of mussel shells (*Mytilus californianus*). We have even observed a number of juveniles occupying space directly on the shells of much larger *L. gigantea* individuals; seemingly trying to occupy any space outside of their home range. Given that large territory holders create such unfavorable environments for smaller non-territorial individuals (i.e. small males and juveniles), we hypothesize that size-selective harvesting indirectly favors these indviduals. One way to test this prediction is to exclude juveniles from our abundance estimates and consequently test whether protected and exploited sites have significantly

different adult abundances. On average, juveniles represent over a quarter (26%) of the individuals per m^2 at exploited sites (5.33 individuals per m^2; SD 1.71; n=7). In contrast, juveniles make up an average of only 11% of the individuals per m^2 at protected sites (1.74 individuals per m^2; SD 0.853; n=3). When juveniles are excluded from our estimates, abundance at protected and exploited sites do not significantly differ (KS test p=0.499). Thus, the increased number of juveniles at exploited sites appears to be a major reason why exploited sites have higher overall abundance of *L. gigantea*, despite harvesting pressure.

Similar differences between exploited and protected sites have been documented in another size-selectively harvested sex changing limpet species in South Africa. The abundance and recruitment of juveniles of *Cymbula oculus* was observed to be three times higher at exploited sites and inversely correlated with adult densities (Branch & Odendaal 2003), which were partly attributed to the negative impact adults have on the survival of juveniles (e.g. through bulldozing). Although we did not directly measure recruitment for our study, a negative correlation between the mean proportion of juveniles per m^2 and the mean size of adults across sites further supports the hypothesis that large individuals at protected sites create unfavorable environments for juvenile *L. gigantea* (Fig. 2-11). However, other studies have found that the adult composition of a population can have either no effect or even a positive effect on the abundance and recruitment of juveniles (Branch 1975; Creese 1981; Quinn 1988). Thus, the effects of size-structure on overall abundances can be system specific.

Although exploited sites have significantly higher abundances of *L. gigantea* compared to protected sites, this difference does not lead to significant increases in overall biomass or reproductive output. Increased abundance at exploited sites is partly a result of the increased numbers of juveniles. These individuals have a minimal effect on the biomass and no effect on the reproductive output of the population. Whether high levels of biomass and reproductive output at protected sites result in their disproportional supply of recruits to exploited sites is not known, but is currently a subject of great interest in marine conservation biology (Halpern & Warner 2003; Parnell et al. 2005; Guidetti & Sala 2007). However, recent genetic analyses (Fenberg et. al. in prep) indicate that there are high levels of gene flow among populations and no genetic structure exists between populations spanning our study area. Regardless of where recruits are coming from, their chance of survival will likely be higher at exploited sites due to the lower overall numbers of one of their main competitors, large territorial females.

The historical effects of size-selective harvesting

Maximum size of *L. gigantea* individuals living along the Palos Verdes peninsula has declined by nearly one-third of their historical level over the last 100 years (90.3 mm in 1904 – 66 mm in 2005). This estimate of size-decline is on par with the modern maximum size difference between the Palos Verdes sites and our other well-protected sites (avg. max size for Palos Verdes populations = 65.6 mm; avg. max size for well-protected populations = 94.3 mm) suggesting that the historical decline in size is largely a

consequence of size-selective harvesting. Because there are no latitudinal trends in body size within our study region, these observations also suggest that Palos Verdes populations in the past were, in all likelihood, similar to the few well-protected populations found today elsewhere along the California mainland. However, we are not able to make direct comparisons of overall size-structure because museum data are time-averaged and the collections are not likely to be an unbiased representation of all size-classes historically present. Nonetheless, we can infer historical changes by comparing modern ecological differences between Palos Verdes sites and well-protected sites (see below).

Ecological effects (historical and modern)

In addition to its effects on the size-structure, abundance, biomass, and reproductive output of the target species, size selective harvesting can also have cascading effects on the other species living in the same community. Many of the studies examining the effects of size-selective harvesting on marine ecological communities involve rocky intertidal communities (Siegfried 1994), particularly those in which large grazers, such as limpets, are the target species (Hockey 1987; Godoy & Moreno 1989; Branch & Moreno 1994; Lindberg et al. 1998). The ecological consequences of harvesting depend, at least partly, on the functional role and competitive dominance of the target species (Kaiser & Jennings 2001). Thus, the effects tend to be system specific and potentially quite complex. But in general, selective harvesting of the target species can increase the growth rate, size and abundance of other non-harvested species due to a release from

competitive pressure (Fenberg & Roy 2008).

Experimental removals of territorial individuals of *L. gigantea* have shown that interspecifics such as macroalgae, smaller limpet species and mussels colonize spaces previously occupied by *L. gigantea* relatively quickly (Stimson 1970; Lindberg et al. 1998). In addition, open space in mid-intertidal habitats tends to be more common in communities with large *L. gigantea* (Lohse 1993). These general results are supported by our data showing that significantly less area is occupied by *L. gigantea* individuals in mid-intertidal habitats at exploited sites where large individuals of this species are absent, compared to protected sites with many large territorial individuals (see Figs. 2-6, 2-7). This also indicates that a shift in space-occupancy of mid-intertidal communities has occurred, at least in part, due to size-selective harvesting pressure. In fact, our data also reveal a shift in community compositions between exploited and protected sites with macroalgae, mussels and barnacles taking up significantly more of the mid-intertidal space at exploited sites (58.1%) compared to protected sites (29.2%) ($p<0.0001$). In fact, the mid-intertidal at protected sites can be characterized as primarily *L. gigantea* grazing habitat dominated by microalgae (Connor 1986) (i.e. avg. 62.6% of the space is *L. gigantea* grazing space), and to a lesser extent, filter-feeding organisms (11.1%) and macro-algae (18.1%). In contrast, the mid-intertidal at exploited sites consists of *L. gigantea* grazers and associated microalgae (40.4%), filter-feeders (32.3%) and macro-algae (25.8%). Thus an indirect effect of size-selective harvesting of *L. gigantea* may be a shift in the functional diversity of the mid-intertidal habitat. How much of this change is exclusively due to the cascading effects of *L. gigantea* size-selective harvesting is not

known, and other factors such as environmental change and habitat modifications are also potentially important here.

Considering that sites that are exploited today historically contained large individuals that are commonly present at protected sites, it is likely that harvesting has played at least some role in mediating these ecological changes. For example, the largest individual collected for a museum at Pt. Fermin (one of our Palos Verdes sites) was 89.3 mm from 1917. The largest individual we found in 2005 at this locality was 66 mm. Because there are no significant latitudinal trends in body size, we can infer historical ecological changes at this locality by comparing modern differences of space-occupancy in mid-intertidal habitats at Pt. Fermin with well-protected sites. On average, 41.5% of the mid-intertidal space at Pt. Fermin is composed of *L. gigantea* grazing space (n=12 quadrats; SD 10.2%). Based on our analysis of *L. gigantea* grazing space at well-protected sites (see above), we deduce that the amount of space occupied by *L. gigantea* in the mid-intertidal at Pt. Fermin has declined by approximately 21.1% over the last 88 years (2005-1917).

Apparent shifts in space-occupancy have other effects on the community as well. For example, higher percent cover of mussel beds at exploited sites creates more habitat (Lohse 1993) for individuals that rely on them for protection from desiccation and predators, as well as for settlement substrate for various larvae, including those of *L. gigantea*. This is potentially another reason why juveniles are more common at exploited sites (see above). In fact, nearly half (49%; 68 /139) of the juveniles that we measured at

the Scripps UC reserve, an exploited site, occurred inside mussel beds, whereas only 5% (2/37) of juvenile individuals measured at a protected site (Vandenberg) were found to be within mussel beds.

Other factors besides the cascading effects of size-selective harvesting have also likely contributed to the differences in abundance and size-structure we see today between exploited and protected sites. The main predators of small to medium sized limpets, including *L. gigantea*, are oystercatchers of the genus *Haematopus* (Hockey & Branch 1984), whereas large individuals tend to have a size-refuge from predation (Hockey 1987). Along the west coast of North America, the American Black Oystercatcher, *Haematopus bachmani* is known to prefer *L. gigantea* as a food source (Legg 1954; Lindberg et al. 1987; Lindberg et al. 1998). However, this species is sensitive to human disturbance (Lindberg et al. 1998) and has therefore become increasingly rare or absent at many California rocky intertidal sites, including all of our exploited sites and one of our protected sites (Cabrillo National Monument). Thus, the absence of oystercatchers indirectly favors the probability of survival for the smaller to medium size-classes, providing further support of why these individuals are very common at exploited sites.

Summary

In general, sites where *L. gigantea* is size-selectively harvested differ significantly in terms of size, abundance, biomass, and reproductive output from those populations of

this species that are protected from human harvesting. These differences also lead to changes in the structure of the mid-intertidal communities, primarily due to the scaling relationship between size and space occupancy in *L. gigantea*.

Exploited sites consist of many small to medium sized individuals but lower biomass and reproductive output, whereas protected sites also contain these size classes, but they tend to be spatially dominated by larger individuals with lower overall abundances. Larger individuals take up proportionally more space exclusively for themselves, causing protected populations to be composed of fewer individuals per unit area compared with exploited sites. Smaller individuals take up less space; thus, the capacity for more individuals to occupy a given area is increased at exploited sites. Large individuals create unfavorable environments for small adults and juveniles due to their highly territorial behavior, thus, exploited sites with fewer large individuals and smaller territories are indirectly favorable to the smaller size classes. The removal of highly territorial individuals relaxes competitive pressure, allowing other space-competitive species to colonize previously occupied territories. Such a relaxation of competitive pressure causes shifts in space occupancy and ecological functioning in the mid-intertidal, and may indirectly contribute habitat for recruiting *L. gigantea* juveniles. Other factors, such as reduced predation by oystercatchers may have also contributed to these modern patterns of size-structure and abundance at exploited sites.

Conclusions and management recommendations

The results of our study show that size-selective harvesting may have indirect effects that can potentially be construed as being good for *L. gigantea* and their surrounding community (i.e. increased number of juveniles; higher mussel densities). However, from a management perspective, our results are troubling in many respects. Size-selective harvesting not only removes the largest individuals of the target species, but it effectively truncates many aspects of its life history and demography that are inherently linked to the large sizes they have evolved with. For example, in *L. gigantea*, large size is associated with territoriality, increased fecundity, and as a refuge from predation. While removal of large individuals by humans increases the local density of this species, it also leads to a substantial decrease in reproductive output.

It is apparent from our historical data and modern field surveys that most California mainland populations of *L. gigantea* are nowhere near their historical sizes. Reversing this trend of size decline would require proper enforcement of the laws already in place to prevent poaching that has become commonplace even at designated MPA's (personal observation; B. Pister; B. Becker personal communication). In addition, the current bag limit of 35 individuals per day with no size-restrictions (outside MPA's) (CA Fish and Game personal communication) is in desperate need of updating, both in terms of how many can be legally harvested (if any) and where. Future studies will focus on how size-selective harvesting affects the life history and genetic structure of *L. gigantea* populations along the California coast. These ongoing studies will help to inform and implement sound management strategies for this ecologically important species.

Table 2-1. The sites in bold are well protected. Values in parentheses are the standard errors of the mean lengths and abundances for each sampled site.

Field site	Latitude(ºN)	Mean Length(mm)	Max. Length(mm)	Mean Abundance
Pebble Beach	36.56º	50.4 (.97)	97	13.0 (1.2)
Ken Norris U.C.R	35.52º	40.1 (.46)	79	14.1 (8.7)
Vandenberg A.F.B	34.73º	53.5 (1.0)	95	10.4 (5.6)
Abalone Cove	33.73º	31.1 (.67)	66	16.0 (3.0)
S. Whites Pt.	33.71º	30.8 (.32)	65	17.6 (1.3)
Pt. Fermin	33.70º	33.1 (.45)	66	17.4 (2.3)
1000 Steps	33.49º	34.6 (.70)	60	14.4 (1.7)
Scripps U.C.R	32.87º	35.6 (.58)	70	22.2 (1.6)
Cortez	32.82º	39.6 (.32)	78	24.1 (1.4)
Cabrillo N.M.	32.66º	50.0 (.57)	91	12.4 (.60)

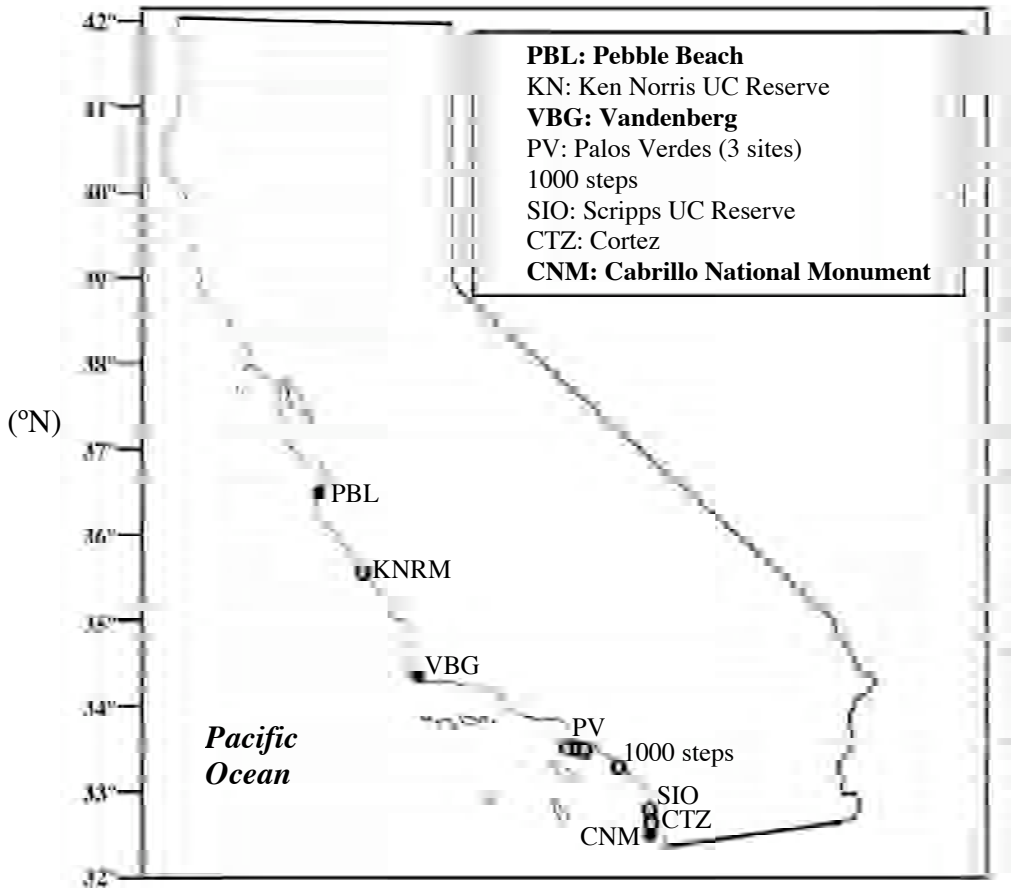
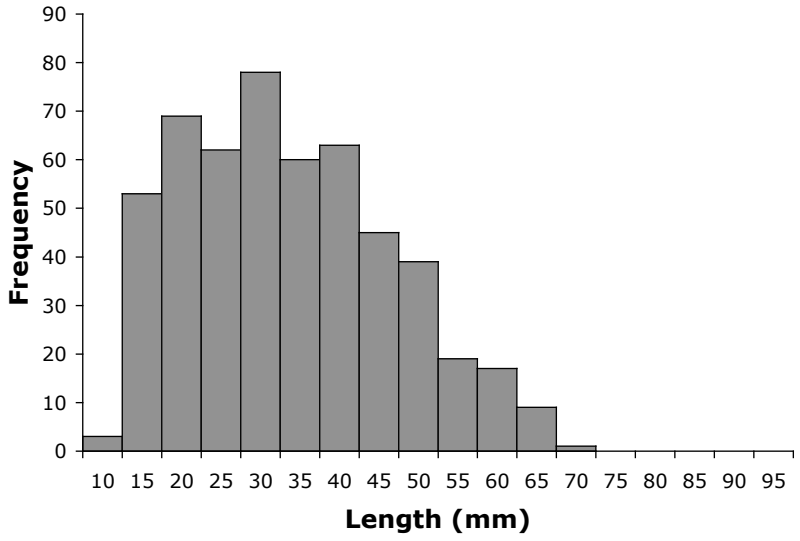


Figure 2-1. Map of California study sites labeled by protected (black circles) and exploited (white circles).

A.



B.

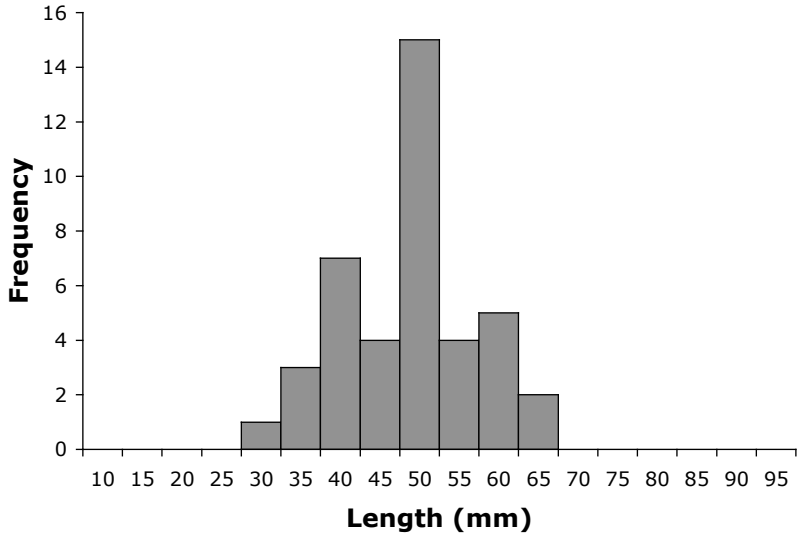


Figure 2-2. Size-frequency distributions of (A) *L. gigantea* individuals from the Scripps UC reserve (mean length = 35.6 mm) and (B) poached individuals (mean length = 50.9 mm) from the same location.

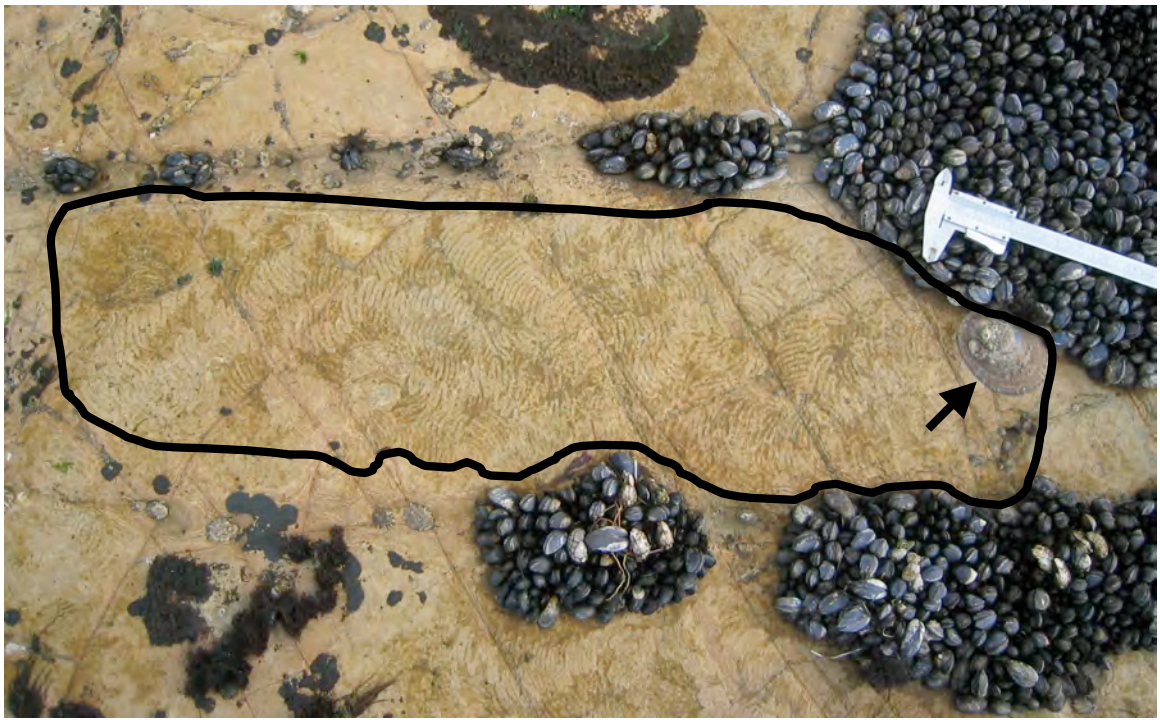


Figure 2-3. Picture of a typical *L. gigantea* territory. The individual (marked by an arrow) is 82 mm long with a territory area of 185,731 mm^2. Inspection of the substrate reveals distinctive radula marks that outline the extent of the territory (outlined with a black line).

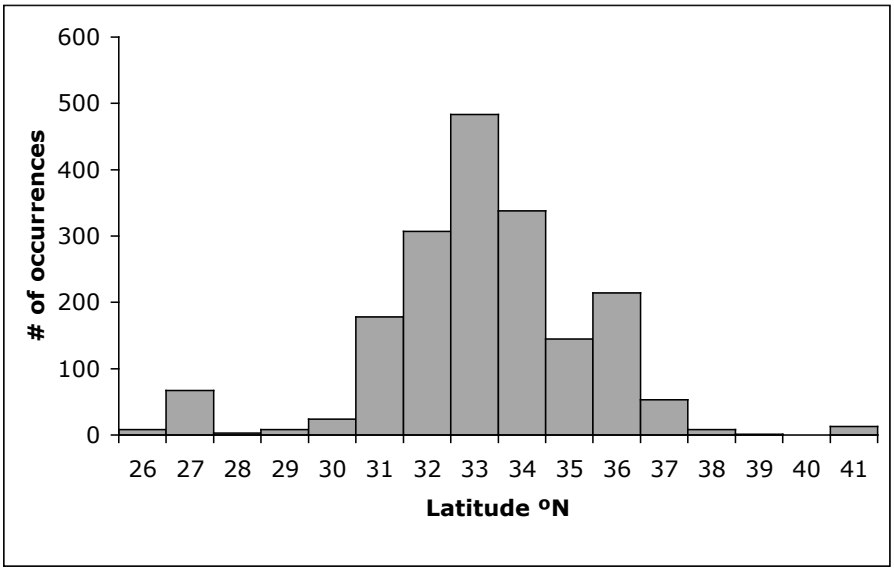
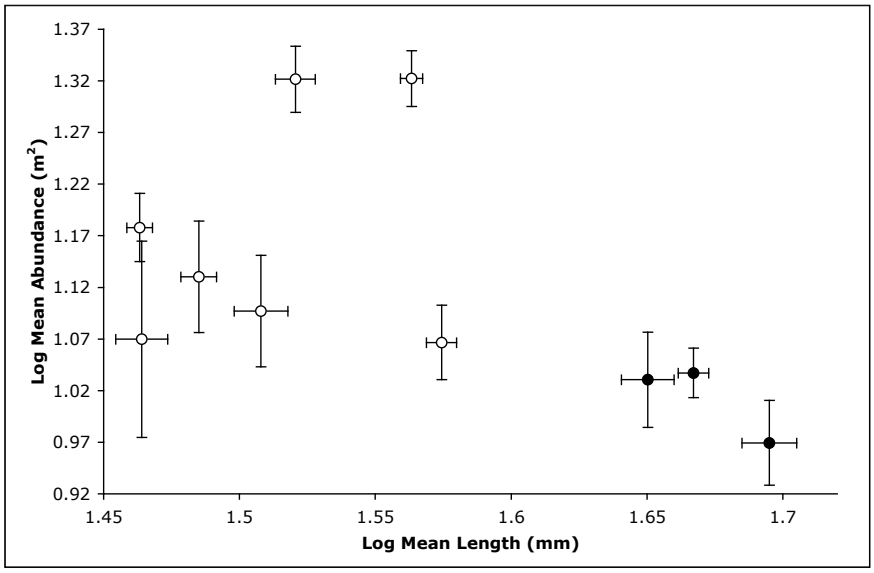


Figure 2-4. Latitudinal frequency distribution of museum collected *L. gigantea* shells from six major natural history museums. Note that the mode of the distribution of occurrences come from 33ºN (Los Angeles county).

A.



B.

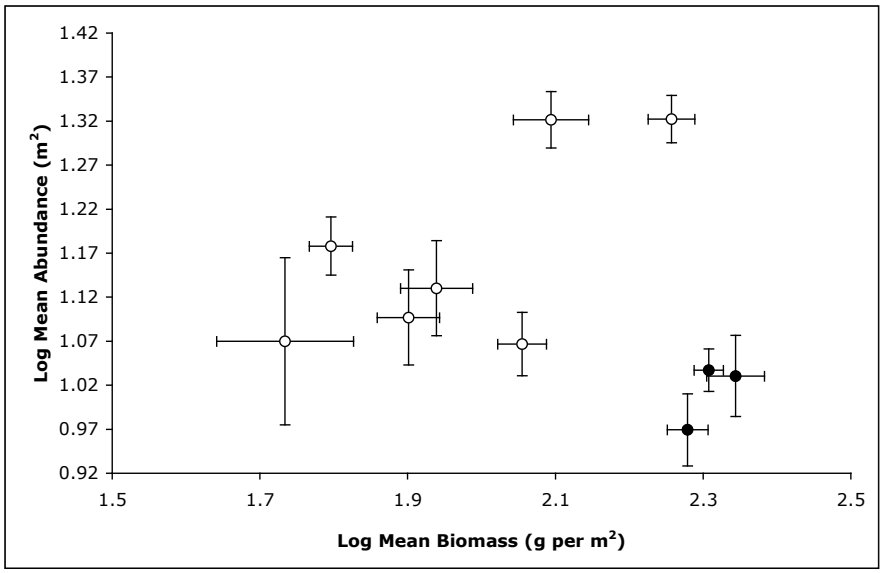


Figure 2-5. Bivariate plots of (A) log mean abundance (individuals per m^2) by log mean length (mm) and (B) log mean biomass (g per m^2). Error bars represent the standard error of the mean and reflect the variance in the abundance, length and biomass per m^2 across each field site. Note that the protected sites cluster together in both plots even though they are separated by 2 – 4º of latitude, indicating that there are no natural geographical trends in abundance, length, or biomass (see text).

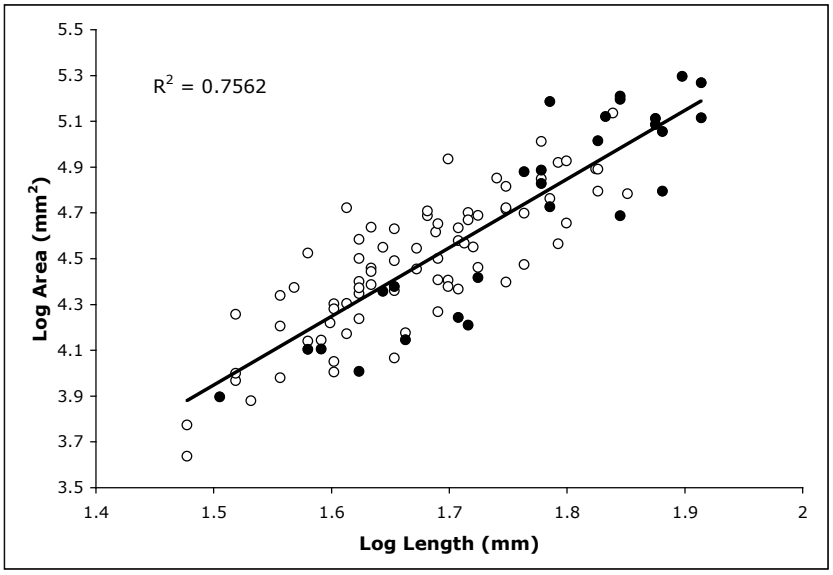


Figure 2-6. A regression of log territory / grazing area (mm^2) by the log length (mm) of the individual occupier (r^2 =0.756; p<0.0001). Black circles represent individuals measured from protected sites and the open circles are individuals from exploited sites. The scaling relationships are not significantly different when regression analyses are performed for exploited and protected sites separately [exploited: intercept = -.20(95%+/-.73) slope = 2.80 (95%+/-.44); protected: intercept = -1.66 (95%+/- 1.17) slope = 3.6 (95%+/-.67)].

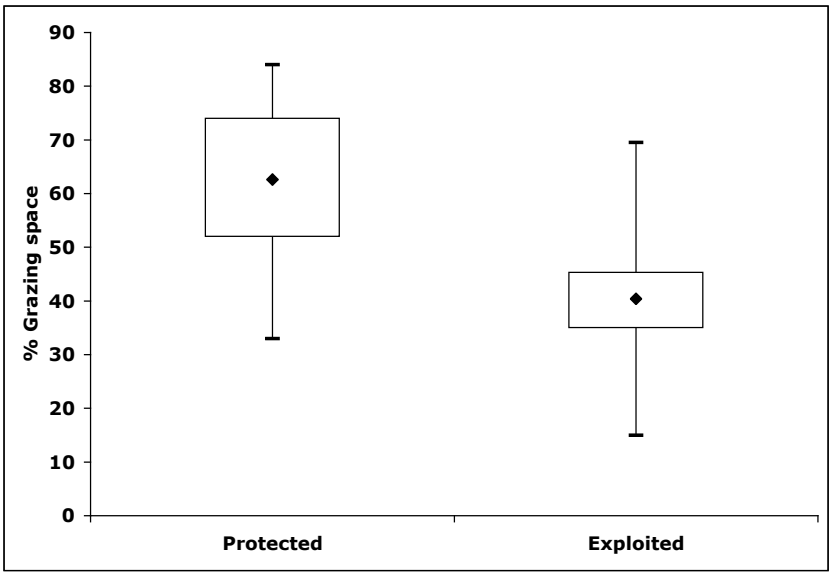


Figure 2-7. Boxplots of the estimated proportion of mid-intertidal area (per m^2) occupied by *L. gigantea* at exploited and protected sites. The values are minimum and maximum, 75% and 25% quantiles and the mean (black diamonds) for 40 quadrats each from exploited and protected sites. There is significantly more area occupied by *L. gigantea* at protected sites (t-test $p<0.0001$).

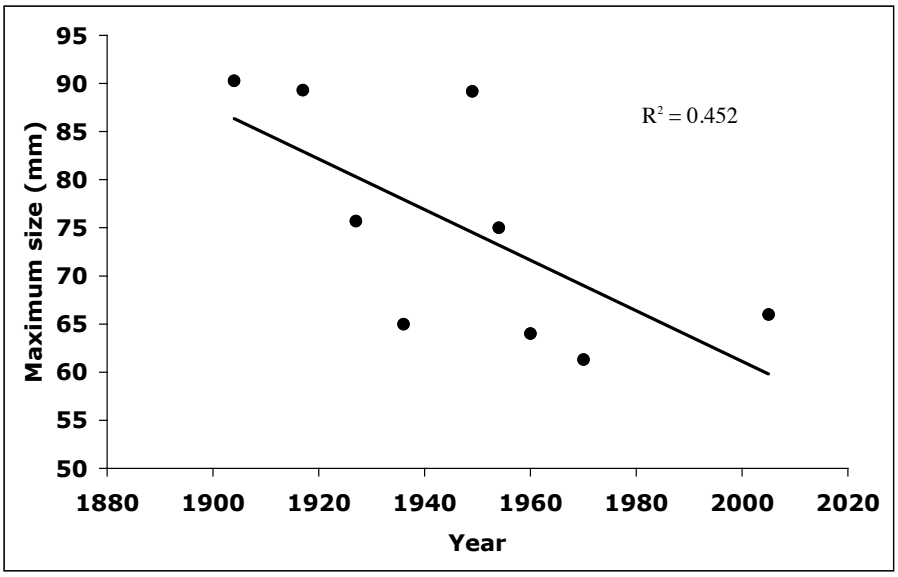
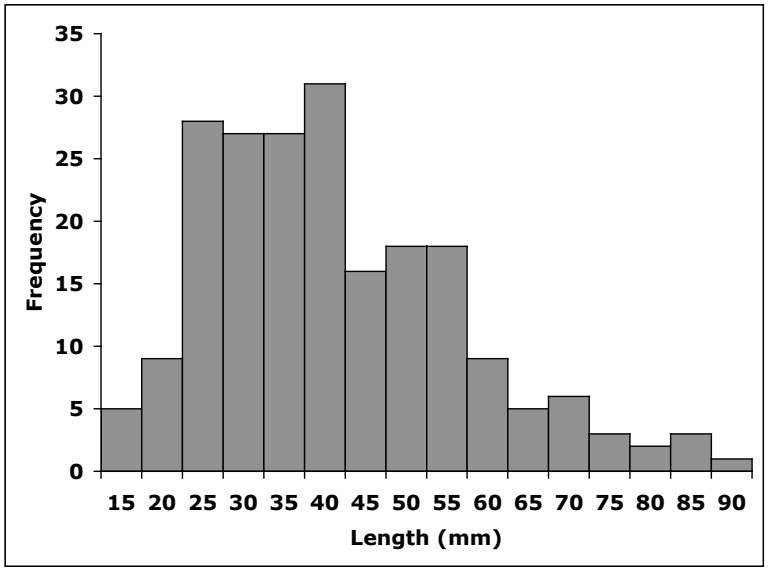


Figure 2-8. A regression of maximum size by year for museum collected *L. gigantea* shells from the Palos Verdes peninsula ($r^2 = 0.452$; $p<0.05$). The last point (2005) comes from our field sampling of three Palos Verdes sites.

A.



B.

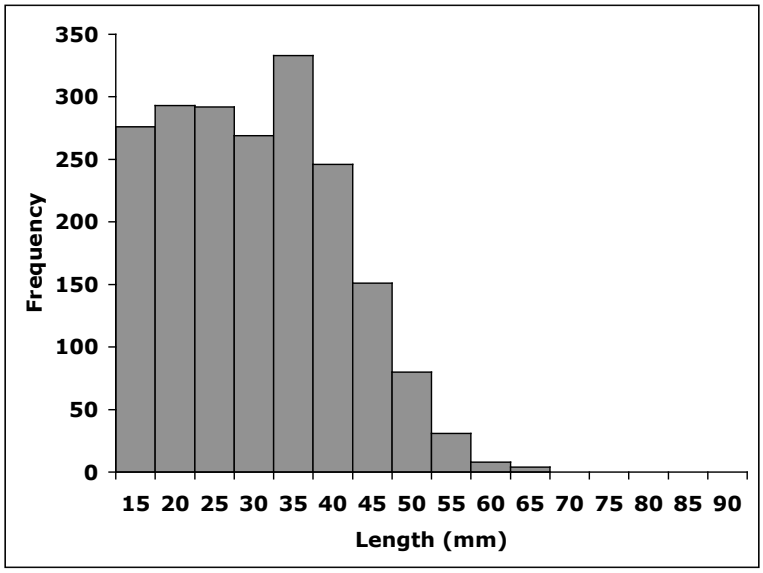


Figure 2-9. Size-frequency distributions of (A) museum shells collected before 1960 from the Palos Verdes peninsula (n=208) and (B) modern individuals measured from the same location (n=2042).

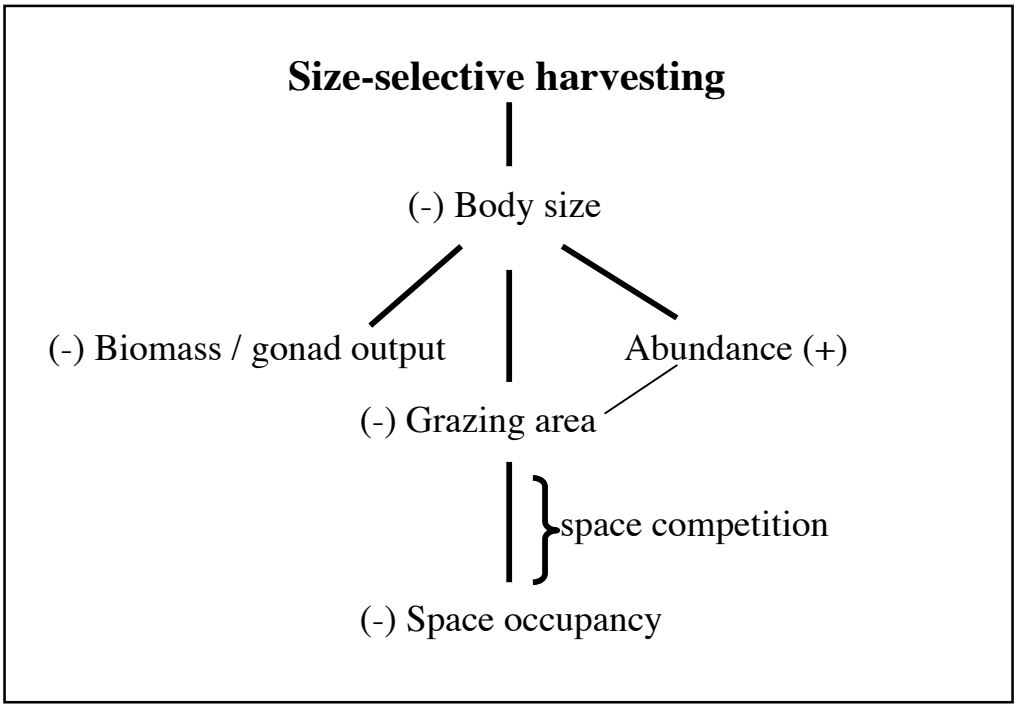


Figure 2-10. A chart of the general effects of size-selective harvesting on *L. gigantea* and their surrounding communities. Harvesting directly reduces the mean and maximum body size of *L. gigantea*, and indirectly reduces biomass / gonad output (per m^2), but increases the total abundance of individuals. Harvesting also removes the most space-competitively dominant individuals (i.e. larger size-classes), thus, relaxing competitive pressure and allowing other space-competitive species to colonize the previously occupied territories. This ultimately results in less space available for the feeding and growth of the remaining *L. gigantea* individuals (in the mid-intertidal).

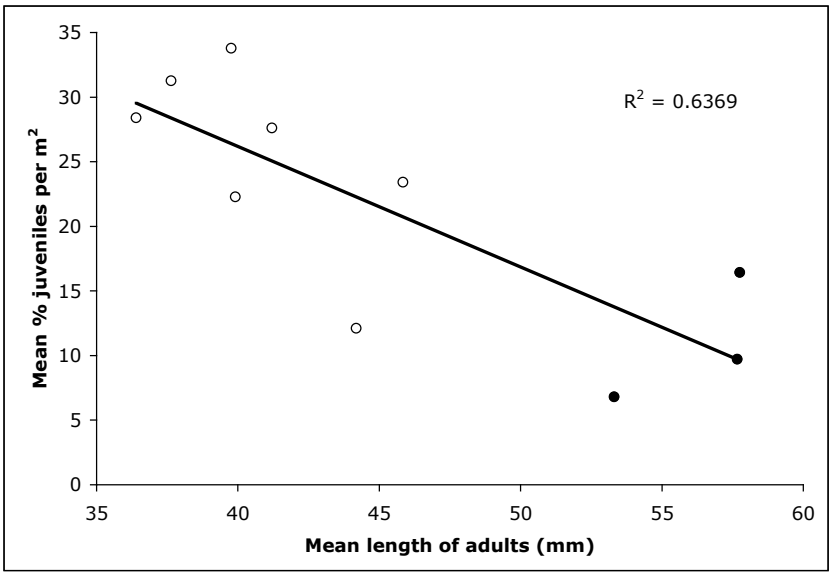


Figure 2-11. A regression of the mean percentage of juveniles (<25mm) per m^2 by the mean length (mm) of adults at protected (black circles) and exploited sites (r^2 = .64, P=.005). Larger individuals create unfavorable environments for juveniles and small adults (through territorial behavior); thus, size-selective harvesting may indirectly increase their chance of survival and lead to higher abundances.

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Chapter 3

The plasticity of life history traits in response to human impacts

Introduction

The selective removal of the largest individuals from a population (size-selective harvesting) is one of the most wide spread anthropogenic impacts of species in the marine environment (Fenberg & Roy 2008). It has therefore become increasingly important to know if and how species are able to respond to such losses. Size-selective harvesting is particularly worrisome for species where the large size classes are composed primarily of one sex, as is the case for sequentially hermaphroditic fish and invertebrates. Sex allocation theory (Charnov 1982), as it applies to sequential hermaphrodites, predicts the timing of sex change given a specific set of demographic parameters, such as the growth rate of individuals, the size and age structure of the population, and adult mortality. If adult mortality rates are unnaturally high and/or growth rates are reduced because of size-selective harvesting, it is predicted that the size (age) at sex change will be reduced in order to compensate for the impacts on breeding sex ratio, and such trends are evident in some size-selectively harvested fish and invertebrate species (Charnov 1981; Cowen 1990; Hannah & Jones 1991; Buxton 1993; Charnov & Hannah 2002; Platten et al. 2002; Hawkins & Roberts 2004). If size at sex change is reduced in harvested populations, then the relative timing of sex change (size at sex change / maximum size) can be predicted to be approximately constant in accordance with more recent advances in sex change theory (Charnov & Skuladottir 2000). However, such a prediction has never been tested using all of the necessary demographic parameters for multiple populations of a sex-changing species. Here, we present results from field measurements on the timing of sex change in

a harvested limpet species that provide demographic support for both a reduction in size at sex change and a constant relative size at sex change.

Coastal marine invertebrates such as the Owl limpet, *Lottia gigantea*, are ideal study organisms to test these predictions because key demographic parameters such as size (age) structure and individual growth rates can be easily measured (see below). *Lottia gigantea*, is a size-selectively harvested species found along the rocky intertidal coastline of western North America (~26ºN-39ºN). Like all molluscs that are able to change sex, *L. gigantea* is characterized by protandric hermaphroditism (Wright 1988). All individuals start their reproductive life as male and change sex to become female as they grow older and larger. Thus, size selective harvesting is likely to preferentially remove the large and older females from the population. A number of studies have documented the effects of size-selective harvesting on this species (Pombo & Escofet 1996; Lindberg et al. 1998; Kido & Murray 2003; Roy et al. 2003; Sagarin et al. 2007), and their universal finding is that selective harvesting causes a decline in the mean and maximum size of affected populations. We will therefore consider mean and maximum body size to be a proxy for human impacts.

A commonly used measure for size at sex change is the length at which 50% (L_{50}) of the individuals are the second sex (Charnov & Skuladottir 2000; Allsop & West 2003b, a; Collin 2006). If growth is well described by the von Bertalanffy growth (VBG) equation ($L = L_{max} (1-e^{-kt-to})$), then the expected value of relative size at sex change (L_{50}/L_{max}) is equal to $1- e^{-kt^{*}-to}$, where k is the growth coefficient (the rate of decrease in

growth with age), t* is the age at sex change, t_0 is the theoretical age at length zero, and L_{max} is the maximum or asymptotic size of the population. Therefore, populations sharing approximately the same L_{50}/L_{max} value should also have a constant kt^*-t_o (Charnov & Skuladottir 2000). However, previous studies have only measured L_{50}/L_{max} across populations and species (Charnov & Skuladottir 2000; Allsop & West 2003a; Collin 2006) without regard to the other parameters (kt^*-t_o).

We have empirically measured all of the VBG parameters (k, t*, t_o, L_{50}, L_{max}) and size/age structure for 10 different *L. gigantea* populations experiencing a wide-range of harvesting pressures (see methods). We predict that size at sex change (L_{50}) will decrease with increasing harvesting pressure (lower L_{max}) but that the ratio (L_{50}/L_{max}) will remain constant across populations. We use three different approaches to test our prediction: 1.) By examining the covariation between L_{50}/L_{max} and other traits considered to be of general importance to life history (e.g. mean body size and age across populations), as suggested by several authors (Nee et al. 2005; Munday et al. 2006; Savage et al. 2006). 2.) Determining whether the observed range of L_{50}/L_{max} values are a particularly constrained subset of biologically relevant values of this trait. And finally, testing whether observed (from field surveys) versus expected values (based on kt^*-t_o) of L_{50}/L_{max} are significantly different. We conclude by discussing the demographic and ecological reasons behind the plastic response in the timing of sex change across populations and the resultant invariance of L_{50}/L_{max}.

We will define L_{50}/L_{max} to be invariant if it does not systematically change across

populations with widely varying body size and age distributions and if it is constrained within a small range of biologically relevant values (Charnov 1993). Body size is the trait directly under selection pressure by humans, and therefore, one that would certainly be correlated with L_{50}/L_{max} if individuals were not able to adjust their timing of sex change in response to size-selective harvesting. If size at sex change were fixed, as it appears to be for some harvested species (Branch & Odendaal 2003), then L_{50}/L_{max} would be negatively correlated with traits such as mean population body size.

Results and Discussion

Our results show that exploited *L. gigantea* populations consistently change sex at smaller sizes compared to protected populations (figure 3-1). The average relative size at sex across all populations is 0.752 (range of L_{50}/L_{max} values = 0.692 – 0.784). Size at sex change (L_{50}) is highly correlated with maximum body size (L_{max}) and hence, susceptibility to harvest pressure across *L. gigantea* populations ($r^2 = 0.932$, $p<0.0001$; figure 3-2). The slope is not significantly different from 1 (0.956 +/- 0.184) when plotted on a log scale. Additionally, L_{50}/L_{max} is not significantly correlated with either mean body size ($p=0.365$) or mean adult age ($p=0.932$) across populations (figure 3-3). In fact, the regressions are essentially flat (slopes not different from zero), which demonstrates that L_{50}/L_{max} does not systematically vary across populations with widely varying size and age distributions. Further, the bias towards the first sex (males in this case) is supported by theoretical predictions (Charnov & Bull 1989), where the average proportion of females (number of females / number of males + females) across each

population is 0.322 (minimum = 0.262, maximum = 0.387), and is not significantly correlated with L_{50}/L_{max} (p=0.868), mean (p=0.933) or max body size (p=0.920), or mean adult age (p=0.220).

The observed values of L_{50}/L_{max} are a particularly constrained subset of what is biologically relevant for this species. Age at maturity (α) is approximately 2 years (based on the youngest males in our dataset), and sex change can occur 1 year after maturity (see above), so the biologically lower limit to L_{50}/L_{max} will be at age 3, which corresponds to a value of .490 (averaged across 10 populations). The upper limit to L_{50}/L_{max} will be at the maximum age recorded for each sampled population, which corresponds to an average of .876. Therefore, the range of possible values for L_{50}/L_{max} is much larger (.386) than the observed range of values (.093). This constrained range of L_{50}/L_{max} values along with the lack of systematic covariation between mean age and body size supports our prediction that relative size at sex change remains constant across *L. gigantea* populations, and fits within the classic definition used to describe life history invariants (Charnov 1993).

Using only age structure and growth rate data (k and t^*-t_o), the average expected value for L_{50}/L_{max} across *L. gigantea* populations is .743 (range of values = .697 - .767), and the observed versus expected values of L_{50}/L_{max} do not significantly differ (paired t-test: p = .114). The fact that these values are not significantly different indicates that age structure and growth rate data can reliably be used to predict the observed relative size at sex change at each population. As predicted by theory (Charnov & Skuladottir 2000), it also indicates that the product of k and t^*-t_o is approximately constant (mean = 1.36;

range of values = 1.19 – 1.46), which explains how L_{50}/L_{max} remains invariant across populations (figure 3-3), but it does not necessarily indicate why such an observation should occur. To get a better understanding of this, one must take a closer look at how size-selective harvesting affects the ecology and life history of *L. gigantea*.

The primary effect of size-selective harvesting is an overall reduction in body size and an increased mortality rate of the affected populations. While this accounts for why harvested *L. gigantea* populations have a lower L_{max}, why L_{50} should also be reduced is not immediately apparent. One of the most commonly cited indirect effects of size-selective harvesting, at least for fish populations, is a change in individual growth rates (Heino & Godo 2002), and any selective pressure influencing growth patterns will have a strong effect on size-related traits (Stearns 1992; Heino & Godo 2002), such as the timing of sex change. Size-selective harvest may preferentially remove faster growing individuals that reach a harvestable size at a younger age, leaving individuals with a tendency to grow at a slower rate (Conover & Munch 2002). If individuals from harvested populations do grow slower, and sex change is under exogenous control (as it is for *L. gigantea*), then it is predicted that the size at sex change (L_{50}) would be reduced in these populations. This prediction is most easily visualized by plotting the size at sex change (L_{50}) versus the age at which adults reach a particular size shared by all populations (e.g. 40 mm). Our results support this prediction, indicating that populations protected from harvesting pressure not only change sex at a larger size, but that they also grow faster than individuals from exploited populations (Figure 3-4). Besides the selective removal of the fastest growing individuals from a population, other factors

limiting growth potential and its consequent effect on size at sex change are also evident. As an individual grows (regardless of the species), it needs to occupy proportionally more feeding space in order to fulfill its energy requirements for sustained growth (Calder 1984). Therefore, the largest individuals require the largest amount of area for feeding. Not only are these individuals predominantly female, they are also highly territorial of their feeding space (bare rock covered with a thin layer of microalgae), which they actively patrol by removing any intra (usually smaller males) and interspecific intruders. Selectively harvesting these individuals vacates their territories for other space competitors to colonize (e.g. mussels, barnacles, macroalgae, other limpet species), which effectively decreases the total amount of available feeding area needed for the growth of the remaining *L. gigantea* individuals (Fenberg in prep). Interestingly, evidence also suggests that size-selective harvesting may indirectly increase the total number of *L. gigantea* juveniles and small adults by removing one of their main competitors (territorial females); similar results have been found for other size-selectively harvested sex-changing limpet species (Branch & Odendaal 2003). And since growth in *L. gigantea* is highly density dependent (Wright 1989), any indirect increase in numbers will result in slower overall growth. The combination of these effects (removal of the fastest growers, reduced feeding area, and negative density dependence) will select for slower growth, which can indirectly contribute to a reduced size at sex change (L_{50}).

However, size-selective harvesting may have a more direct influence on size at sex change by affecting the mortality schedule of territorial females. For *L. gigantea*, territory acquisition is considered to be one of the primary mechanisms of sex change

(Wright 1989; Shanks 2002). Males will raid female territories and eat as much microalgae as they can before being pushed off by the larger patrolling female with the anterior portion of her shell (Stimson 1970; Shanks 2002). Size-selective harvesting increases the mortality rate of territorial females above background levels; which opens up territory space for the surrounding males to compete for amongst themselves. These remaining *L. gigantea* individuals may use this initial change in density as a cue to assess local mortality rate (Wright 1988, 1989). The larger (older) males are the best competitors and the most likely to acquire the open territory and change sex, earlier than they would under normal circumstances (Wright 1988, 1989). A negative correlation between size at sex change (L_{50}) and total mortality rate (z) across populations provides support for this prediction ($r^2 = 0.65$; $p = 0.0046$). But whether individuals are directly responding to growth, mortality or the size-distribution of other individuals (a pattern generated by growth and mortality) is not entirely clear, but the observed patterns are a likely result of a combination of these factors.

When the sex change of an individual is under exogenous control, as it appears to be for many species (Munday et al. 2006), then the sex change schedule of the population can change over a short time period in response to demographic fluctuations (Warner 1975). The labile response to the local ecology and demographics that we report in this study indicates that there is not a single optimal size or age at sex change for this species as a whole. Yet individual plasticity can generate significant population-level structure in the relative timing of sex change (e.g. $L_{50}/L_{max} \sim .75$) in response to changes in demography (Warner 1975; Munday et al. 2006).

Size-selective harvesting is an increasing problem for populations in marine environments around the globe (Fenberg & Roy 2008). Given adequate demographic information however, we can test predictions on how populations should respond to such impacts. We have shown that populations of a sex-changing species (*L. gigantea*) respond to demographic changes caused by size-selective harvesting by altering their size at sex change in a predictable manner according to life history theory (Ghiselin 1969; Warner 1975; Charnov 1981; Charnov & Skuladottir 2000). However, this compensatory response may not occur for some species if harvesting pressure is intense enough not to allow adequate time for sex change (Coleman et al. 1996; Hawkins & Roberts 2004). Additionally, if size at sex change is fixed (Branch & Odendaal 2003; Munday et al. 2006), then a smaller size at sex change is not likely under harvesting pressure.

The importance of large and old individuals for populations and ecosystems cannot be overstated (Birkeland & Dayton 2005); and the plastic response of life history traits to size-selective harvesting that we report in this study certainly does not diminish their importance. The larger (older) individuals are the most fecund, produce the highest quality offspring, are the best intra and interspecific competitors, and generally are the best predictors for the overall health of a population (Birkeland & Dayton 2005). The preservation of the natural size and age structure of populations is critical to the conservation and management of species and ecosystems.

Materials and Methods

Field sites

We sampled a total of 10 field sites (populations) for this study. Three sites are characterized as having little if any harvesting pressure (Cabrillo National Monument, Vandenberg Air Force Base and Pebble Beach), and the remaining 7 sites are characterized as being subjected to moderate to high levels of harvesting pressure (SI table 3-1). The field sites range in latitude from San Diego (32.6ºN) to Monterey, California (36.5ºN). This middle portion of the geographic range of *L. gigantea* has a high average abundance and consistent yearly settlement of juveniles. Each site is considered to be a separate population because they are separated by large stretches of unsuitable habitat, and adults do not migrate from the area where they have settled as juveniles.

Sampling design

We measured size-frequency distributions (SFD) for each site by laying down belt transects of meter square quadrats in areas of suitable *L. gigantea* habitat. Using vernier calipers, we measured the longest shell length of each *L. gigantea* individual to the nearest millimeter. We took special care to measure every individual in the quadrats in order have an accurate measure of the SFD for each population. We measured approximately 400-1000 individuals for each site over the course of 2-3 days during the

fall and winter low tides from 2002-2005. We then calculated average abundances per meter^2 and average lengths.

We sampled *L. gigantea* individuals from the same areas where we collected the SFD data (usually during the same low-tide series). Because there are no secondary sexual characteristics available to differentiate males from females, we only sampled during the fall and winter months when *L. gigantea* populations experience a single spawning event (Daly 1975; Kido & Murray 2003), and gonads can be inspected for eggs or sperm. We intentionally sampled individuals proportionally from the population level SFD (i.e. the mode(s) of each SFD were sampled proportionally more than the tails, although all size classes over 20 mm were included in the sampling effort). We deemed this to be a more appropriate sampling scheme from one that is purely random because it better represents the true distribution of genders across each size class. We removed 31-97 limpets from the substrate at each site using a putty knife with a rounded edge and dissected and examined them for eggs (greenish) or sperm (whitish) in the laboratory. We sampled individuals almost exclusively from substrate with relatively low rugosity in order to minimize the effect of habitat type on growth rates (Kido & Murray 2003).

We used binomial gender data criteria (male =0, female =1) to fit a logistic curve of the proportion of females against the body size distribution (Licandeo et al. 2006) for each sampled population using JMP software. We determined the size at sex change (L_{50}) to be the length at which 50% of the individuals are female (Charnov & Skuladottir 2000; Allsop & West 2003b, a; Collin 2006). We tested the suitability of each regression

by comparing the log-likelihood x^2 from the fitted model to a model that assumes there is a random chance of an individual being male or female (Platten et al. 2002). A significant value would indicate that the fitted logistic regression provides a better description of the sex distribution (Platten et al. 2002). The overall fit for each regression was significant ($p < 0.01$; SI table 3-3; SI figure 3-1). We calculated the overall proportion of females at each site to be the number of females / number of males + number of females. In order to avoid sampling juveniles that have no observable sexual characteristics, we restricted our sampling only to individuals over 20-25mm.

Our sampling efforts at the protected sites (Cabrillo National Monument, Pebble Beach, and Vandenberg Air force Base) were slightly reduced (SI table 3-1) compared to the less protected sites for several reasons. First, we wished to minimize the impact to these populations because they represent the highest modern concentration of large individuals (i.e. most fecund) found along the California mainland. Sites like these are rare and therefore should not be subjected to intense scientific sampling. Second, protected populations may be an important source of larvae for less protected areas. And lastly, exploited sites contain on average more individuals per meter square than do protected sites (Fenberg in prep). A plot of L_{50} versus sample size (n) across populations was not significant ($p = 0.316$).

Rather than relying on an extreme value based on only one individual for L_{max}, we decided to take a more conservative approach and used the average of the largest 5% of the SFD as a measure of L_{max} for each population. There are a number of advantages to

this approach. The largest individual in a population can be a numerical outlier from the rest of the SFD (by as much as 10mm) and is often physically isolated from other individuals (most commonly observed at exploited sites). But perhaps most importantly, the largest individual in a population does not adequately estimate the maximum size (L_{max}) predicted by growth rate measurements using the VBG equation (see below).

Growth rate and age determination

We measured growth rates for three separate *L. gigantea* populations from San Diego, CA (Cabrillo National Monument, CNM, Scripps reserve, SIO, and Cortez street, CTZ) by measuring the lengths of tagged individuals every 2-3 months for 1 year. We quadruple tagged individuals with colored and numbered plastic bee tags (The Bee Works) affixed to the shells with super glue. 45-60 individuals spread across the SFD were tagged at each site. We took special care to include individuals from all size classes over 20 mm in order to avoid biases in growth rate and age determination. We were careful to limit our measurements only to individuals living within the same area (~ 5 m^2) in order to reduce habitat related biases in growth (Wright 1989; Kido & Murray 2003). After 1 year of growth, we plotted Ford-Walford plots (Walford 1946) of final size (mm) versus initial size (mm) to obtain estimates of the von Bertalanffy parameters k (natural log of the slope of the Ford-Walford plot) and L_{max} (y-intercept / 1- slope). The regressions for each population are tightly correlated (SI table 3-4), and this method has been applied successfully in other *L. gigantea* studies and for other limpet species in general (Daly 1975; Balaparameswara Rao 1976; Branch 1981; Guzman & Rios 1987;

Kido & Murray 2003). The estimate of L_{max} using this method accurately represents the largest sizes obtained at each population (i.e. average of the largest 5% of the SFD). In addition, the k values (avg. = 0.165) are quite similar to other *L. gigantea* studies (avg. across 3 studies = 0.176) that have used the same methodologies (Stimson 1968; Daly 1975; Wright 1985). Using the VBG equation, $L = L_{max} (1-e^{-kt})$, we then plotted growth curves at these three populations to estimate the ages of the tagged individuals (SI figure 3-2). One potential limitation of the Ford-Walford approach to estimating VBG parameters is that the theoretical age at length zero (t_0) cannot be calculated and is therefore usually assumed to be zero. Since individuals settle on the substrate at such a small size and at a very young age, we will assume that t_0 is negligible and not significantly different from zero when estimating the VBG parameters for the tagged individuals. We will however estimate t_0 when plotting growth curves through length at age data for individual shells sectioned for sclerochronology analysis (see below).

Sclerochronology

The use of growth increments and shell microstructure has played an important role in molluscan ecological, taxonomic, paleobiological, demographic and life history studies (Frank 1975; Lindberg 1986; Harrington 1987; Goodwin et al. 2001; Gilman 2007). When an *L. gigantea* shell is sectioned along the axis of maximum growth (i.e. the longest length), small growth increments can be seen within the M-1 layer of the shell (see SI figure 3-3 and (MacClintock 1967) for definitions of patellogastropod shell layers and microstructure).

The shells from the individuals that we sampled for gender / size distributions at the three San Diego sites were covered in epoxy (JB Kwik) and sectioned along their longest length using a Buehler Isomet low speed saw. We collected these limpets from the same vicinity where we tagged individuals in order to confirm that growth rates from the field match with growth rates from sclerochronology (i.e. from growth increments). We polished each shell section using two successive grit sizes (400 and then 1000) and examined the increments under a dissecting microscope. We recorded the average number of growth increments for 2-3 separate counts for each shell. A small number of shells were heavily eroded, and thus were discarded from the analysis due to the difficulty of counting increments near the shell apex.

To determine if the growth increments are laid down annually, semiannually or more often (Branch & Odendaal 2003), we plotted size versus the number of growth increments directly onto the growth curve plots determined for the tagged individuals, as described above. We found that semiannual (i.e. laid down twice a year) growth increments best fit the predicted growth rate measurements from the tagged individuals (SI figure 3-2). To confirm this, we compared the average age specific lengths for each age group (1 year) obtained through sclerochronology analysis (using 2 growth increments per year) with what is predicted from the VBG equation for the tagged limpets at each population. We then performed a paired t-test to determine if the observed (growth increments) and predicted (from tagged limpets) length at age measurements are significantly different from each other. In addition, we fit a linear

regression through the observed versus predicted values and tested whether the slopes are significantly different from 1. A tightly correlated regression with a slope not significantly different from 1 would indicate that these two methods of determining length at age are equivalent.

The observed versus predicted values of length at age are not significantly different at the three San Diego populations and regressions of observed versus predicted values are tightly correlated with slopes not significantly different from 1, indicating that growth increments can reliably be used to predict the age of *L. gigantea* individuals (SI figure 3-2, SI table 3-5).

Once we determined that growth increments can be used to age individuals, we used non-linear regression to fit the VBG equation through length at age data obtained from sclerochronology analysis for each of the 10 sampled populations. When plotting growth curves for each population, it is important to note that we only used the largest 5% of the SFD as a measure for L_{max}. We believe this to be the best estimate for maximum size because it is a directly measurable quantity of any population and is a consistent measurement for the denominator of field measurements for observed relative size at sex change (L_{50}/L_{max}). To test the suitability of the VBG model, we measured the average observed (from growth increments) and predicted (from the VBG equation) lengths for each age group (1 year) and tested whether they are significantly different from each other using a paired t-test. A non-significant p-value would indicate that the VBG model is a good predictor for the growth of *L. gigantea* individuals. The observed

versus predicted values of length at age are not significantly different at each of the 10 sampled populations (SI table 3-6).

The growth rates predicted both from the tagging study and by sclerochronology analysis closely match what has been observed for other *L. gigantea* studies (based only on tagged individuals (Daly 1975; Kido & Murray 2003); this is corroborating evidence that this species is relatively slow growing and can reach ages of approximately 15-18 years at protected populations and approximately 10-13 years at exploited sites in southern and central California. In addition, the average k values from the tagged individuals from the three San Diego populations (avg. = 0.1643) are nearly identical to the k values obtained through sclerochronolgy analysis from the ten sampled populations (avg. = 0.1649)

To determine the age at sex change (t*) from each population we used the same logistic regression methodology described above for length at sex change. t* is the age at which 50% of the individuals sampled are female (SI table 3-1; SI figure 3-1). Mean adult age (2 years and above) was then calculated at each population (SI table 3-7).

Mortality rate (Z)

We estimated the total mortality rate (Z) using the VBG equation and the length frequency distribution for each *L. gigantea* population. We converted the length data to relative age using the inverse of the VBG equation ($t = \ln (1-L/L_{max})/-k-t_o$) (King 1995).

Z was estimated for adult limpets (over 2 years) from the slope of the linear regression of the natural logarithm of the change in age frequency by the relative age (using 1 year intervals) (King 1995; Platten et al. 2002). We excluded data points close to L_{max} (within 5 mm) because of the uncertainty between length at age for these individuals (King 1995). The total mortality rate (Z) is the sum of the instantaneous harvest mortality rate (F) and the instantaneous natural mortality rate (M). Our data can therefore not be used to separate harvest mortality rate (F) from natural mortality rate (M). However, assuming equal natural mortality rates (M), populations with a high Z should also have an increased value of F. Our results support this prediction (table 8), with exploited sites (presumably high F) having higher mortality rates (Z). Moreover, a negative correlation between size at sex change and Z supports our prediction that harvested populations change sex at a smaller size, which is consistent with what is predicted from life history theory (SI figure 3-4; SI table 3-8).

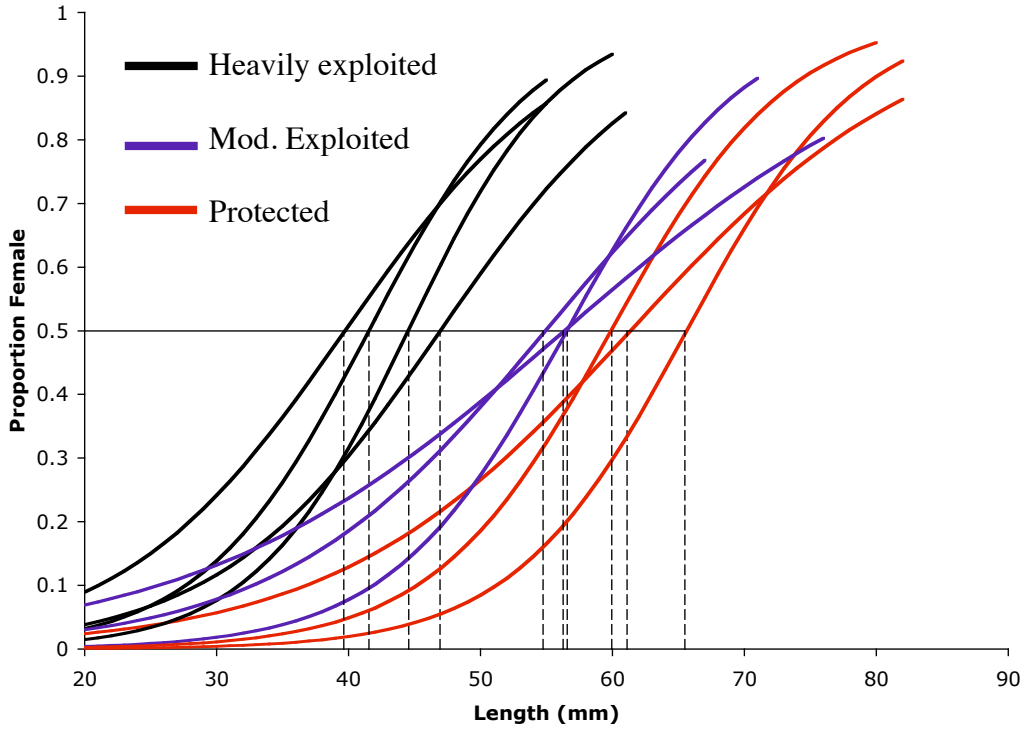


Figure 3-1. The logistic regression curves of the proportion of females against the body size distribution of sampled individuals. The size at which 50% of the individuals are female is the size at sex change (L_{50}). The black curves are from highly exploited populations; purple curves are for moderately exploited populations; red curves are from well-protected populations.

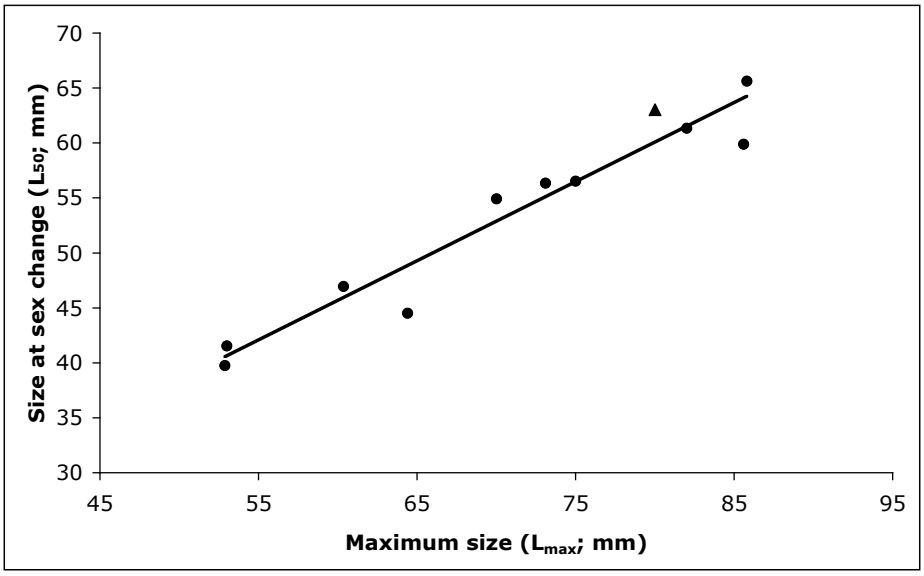
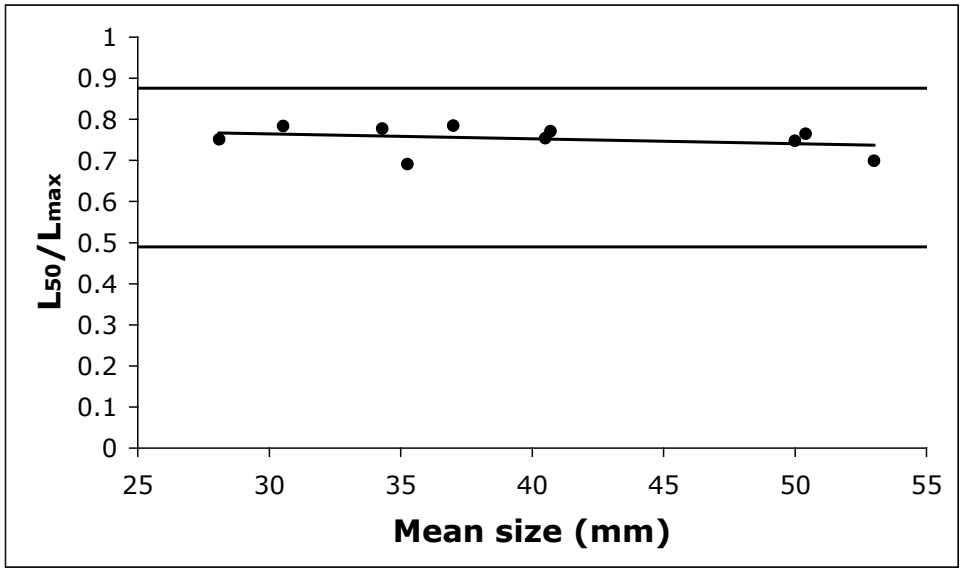


Figure 3-2. The relationship between size at sex change (L_{50}) and maximum size (L_{max}) for 11 *L. gigantea* populations from southern and central California ($r^2 = 0.934$; $p<0.0001$). The point marked by a triangle comes from data collected by (Wright & Lindberg 1982).

A.



B.

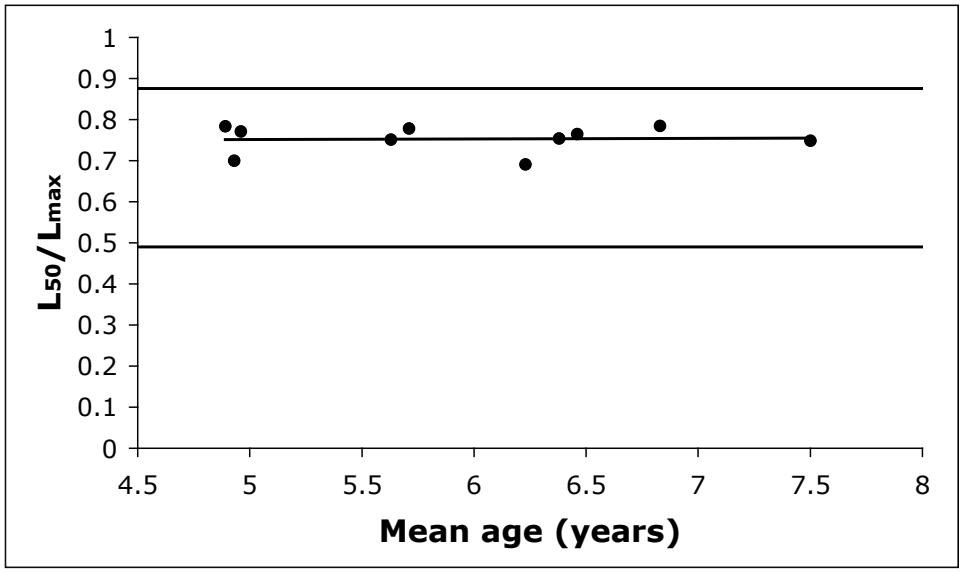


Figure 3-3. Relative size at sex change (L_{50}/L_{max}) for 10 *L. gigantea* populations plotted against mean body size (A) and mean adult age (B). The lower solid black lines (0.490) represent the L_{50}/L_{max} if individuals changed sex at 3 years of age (the earliest plausible age at sex change). The upper solid black lines (0.876) represent the L_{50}/L_{max} if individuals changed sex at the maximum-recorded age for each population. These plots show that L_{50}/L_{max} are a particularly constrained subset of biologically relevant values that do not vary systematically with either mean body size (p=0.365) or mean adult age (p=0.932).

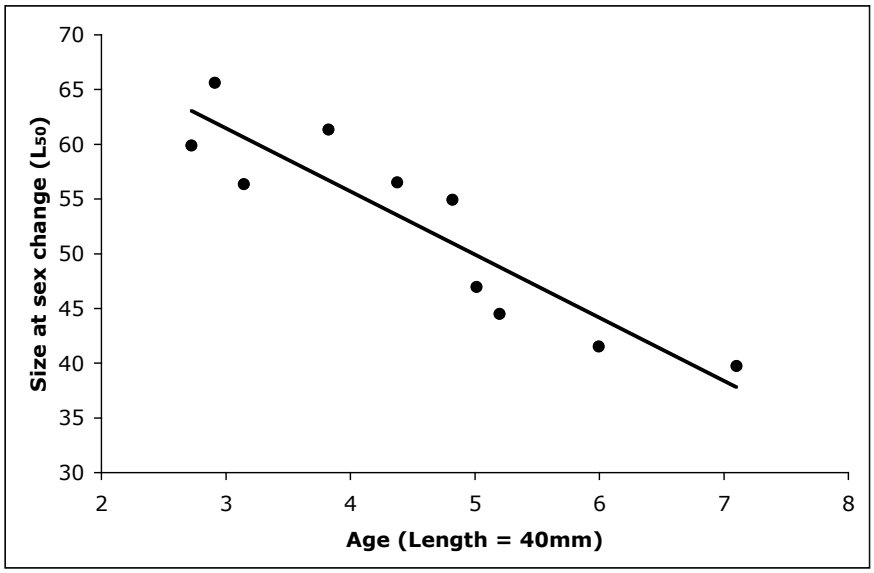


Figure 3-4. A plot of size at sex change (L_{50}) versus the age at which individuals reach a length of 40 mm across 10 *L. gigantea* populations (r^2=0.831; p<0.001), indicating that populations that change sex at a larger size also grow at a faster rate.

Supplementary Information

SI Table 3-1. The sites in bold have zero or very minimal harvesting pressure as indicated by their large maximum sizes. The values of L_{max} are the largest 5% of the SFD at each site. The values of k and t_0 were calculated by fitting the VBG equation through the length at age data using non-linear regression. The L_{50} and t^* values were obtained from the logistic regression curves in figure 1. The data for the San Nicolas Island site are from (Wright & Lindberg 1982).

Field site	Lat. (ºN)	L_{50}	L_{max}	t*	k	t_0	% female	n
Cabrillo N.M.(CNM)	32.66	61.4	82.0	8.59	0.163	-0.292	0.387	31
Vandenberg (VBG)	34.73	59.9	85.6	5.96	0.175	-0.885	0.282	39
Pebble Beach (PBL)	36.56	65.6	85.8	7.06	0.199	-0.236	0.323	31
San Nicolas Island*	33.25	63.0	80.0	NA	NA	NA		
CTZ(1)	32.82	56.5	75.0	8.99	0.144	-0.920	0.278	97
Ken Norris (KNRM)	35.52	56.4	73.1	6.50	0.186	-1.11	0.350	60
CTZ(2)	32.81	54.9	70.0	8.35	0.142	-1.16	0.320	50
Scripps (SIO)	32.87	44.5	64.4	7.08	0.148	-1.36	0.316	57
Pt. Fermin (PF)	33.70	46.9	60.4	6.84	0.157	-1.90	0.324	68
Whites Point (WP)	33.71	41.5	53.0	6.01	0.162	-3.08	0.262	61
Dana Point (DPRR)	33.46	39.7	52.9	6.31	0.184	-0.597	0.375	50

SI Table 3-2. Observed L_{50}/L_{max} and expected L_{50}/L_{max}. The expected values were obtained by inserting the k, t* and t_0 values into the VBG equation: $L_{50}/L_{max} = 1\text{-}e^{-kt^*-to}$. These values are not significantly different from each other (p = 0.2029), which indicates that growth rate and age structure data can reliably predict the observed relative size at sex change at each population.

Field site	observed L_{50}/L_{max}	expected L_{50}/L_{max}
Cabrillo N.M.	0.748	0.764
Vandenberg A.F.B.	0.700	0.697
Pebble Beach	0.765	0.767
San Nicolas Island*	0.787	NA
CTZ(1)	0.753	0.760
Ken Norris R.M.	0.770	0.759
CTZ(2)	0.785	0.740
Scripps	0.692	0.720
Pt. Fermin	0.778	0.747
Whites Point	0.784	0.755
Dana Point R.R.	0.751	0.719
Average	**0.752**	**0.743**

SI Table 3-3. The log-likelihood x^2 and p-values for the logistic regression curves from figure 1.

Field site	L_{50} x^2	L_{50} p-value	t* x^2	t* p-value
Cabrillo N.M.	10.3	0.0013	13.0	<0.001
Vandenberg A.F.B.	17.0	<0.0001	15.5	<0.0001
Pebble Beach	18.2	<0.0001	15.1	<0.0001
Ken Norris R.M.	10.0	0.0016	12.8	<0.001
CTZ(1)	36.5	<0.0001	35.8	<0.0001
CTZ(2)	8.10	0.0045	14.6	<0.0001
Scripps	21.4	<0.0001	25.8	<0.0001
Pt. Fermin	16.4	<0.0001	27.3	<0.0001
Whites Point	23.7	<0.0001	41.2	<0.0001
Dana Point R.R.	14.8	<0.0001	8.4	0.004

SI Table 3-4. The VBG parameters obtained from the Ford-Walford regression of final size (mm) by initial size (mm) for 1 year of growth for tagged individuals at 3 San Diego, CA populations. When plotting the growth curves from the tagging study, it is important to note that we used the top 5% of the SFD as a measure of L_{max} for each population (see text).

Field site	F-W k	F-W L_{max}	r^2	p-value
CNM	0.165	88.8	0.891	<0.0001
CTZ	0.159	67.3	0.885	<0.0001
SIO	0.169	62.7	0.875	<0.0001

SI Table 3-5. Paired t-test p-values of length at age (using 1 year intervals) obtained through the tagging study (observed) and from growth increments (expected) at 3 San Diego, CA populations. The r^2 values and the slopes (+/- 95% CI) refer to the regression of observed vs. expected at each population. The observed vs. expected values are not significantly different and have a slope not significantly different from 1, indicating that sclerochronology analysis may be used to age *L. gigantea* individuals.

Field site	p-value	r^2	slope
Cabrillo N.M.	0.65	0.94	1.00(+/-0.17)
CTZ	0.28	0.96	0.88(+/-0.14)
SIO	0.23	0.92	0.87(+/-0.20)

SI Table 3-6. Paired t-test p-values of length at age (using 1 year intervals) obtained from sclerochronology analysis (observed) and that predicted from the VBG equation from 10 sampled populations. The observed vs. predicted values are not significantly different at each population, indicating that the VBG equations provide a good fit of the length at age data.

Field site	p-value
Cabrillo N.M.	0.2403
Vandenberg A.F.B.	0.2249
Pebble Beach	0.2111
CTZ (1)	0.6232
KNRM	0.5827
CTZ (2)	0.6668
Scripps	0.8712
Pt. Fermin	0.8288
Whites Point	0.0934
Dana Point R.R.	0.1855

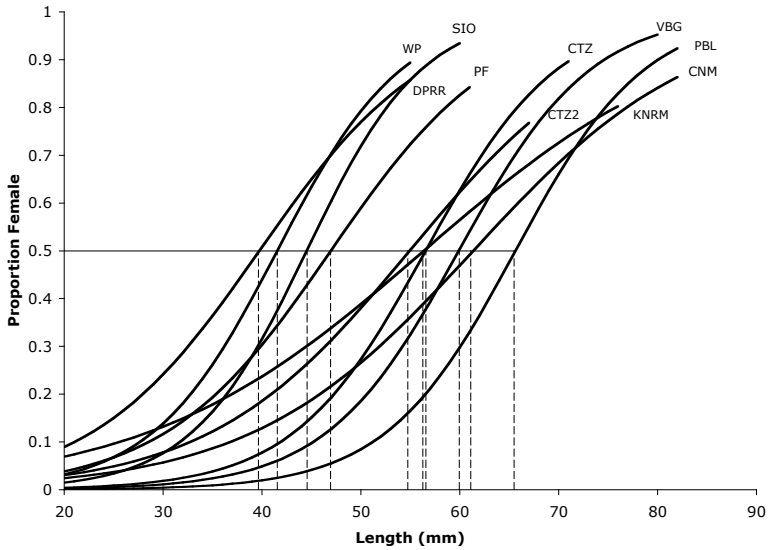
SI Table 3-7. Mean size and mean adult age (above 2 years) for each of the sampled *L. gigantea* populations.

Field site	Mean size (mm)	Mean age (years)
Cabrillo N.M.	50.0	7.50
Vandenberg A.F.B.	53.5	4.93
Pebble Beach	50.4	6.46
Ken Norris R.M.	40.7	4.96
CTZ(1)	40.5	6.38
CTZ(2)	37.0	6.83
Scripps	35.3	6.23
Pt. Fermin	33.1	5.71
Whites Point	32.5	4.89
Dana Point R.R.	28.1	5.63

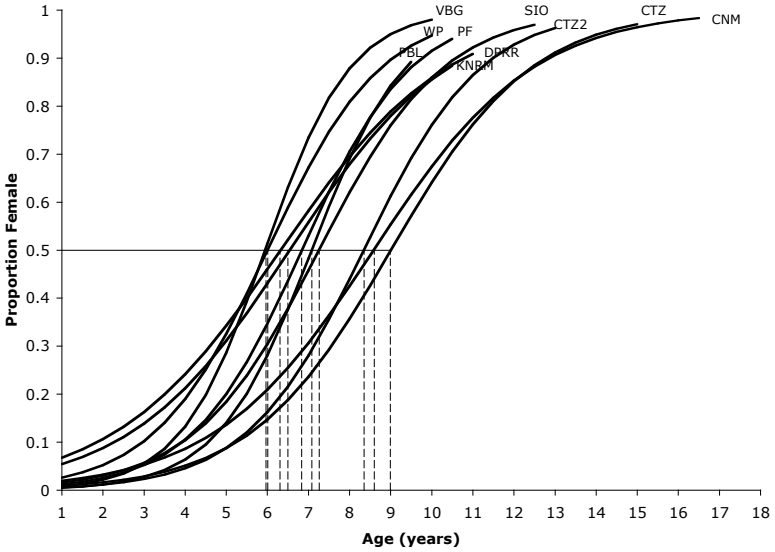
SI Table 3-8. The total mortality rate (Z) for each sampled *L. gigantea* population (see text for description of methods).

Field site	Z
Cabrillo N.M.	0.170
Vandenberg A.F.B.	0.218
Pebble Beach	0.188
Ken Norris R.M.	0.265
CTZ(1)	0.276
CTZ(2)	0.281
Scripps	0.272
Pt. Fermin	0.276
Whites Point	0.283
Dana Point R.R.	0.410

A.

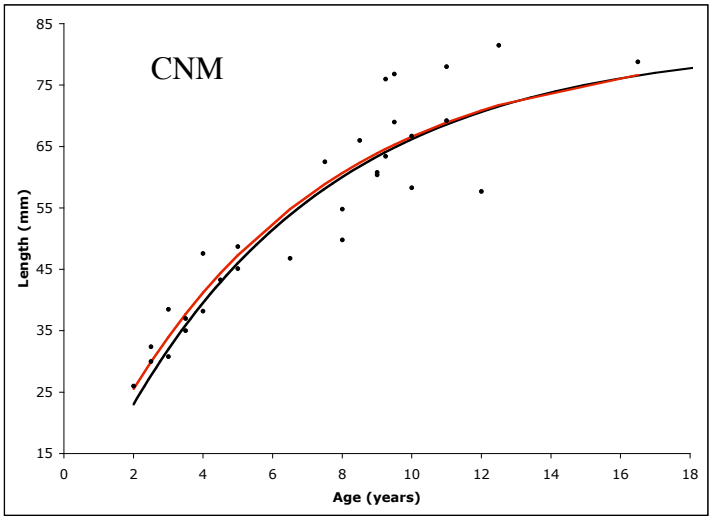


B.



SI Figure 3-1. Logistic regression curves of proportion female by length (A) and age (B) distribution from 10 sampled *L. gigantea* populations. The length or age in which 50% of the sampled individuals are female was used to determine the length (L_{50}) or age (t*) at sex change

SI Figure 3-2. Growth curves for 3 *L. gigantea* populations from San Diego, CA. The black curves represent the length at age estimates for the tagged individuals using the VBG equation (as described in the text). The black dots are the length at age values measured from growth increments for individual shells (i.e. sclerochronology analysis). The red curves represent the VBG equation fit through the growth increment obtained length at age data. The growth curves from the tagging study are not significantly different from the growth curves from the sclerochronolgy analysis at each population, indicating that growth increments may be used to age individual *L. gigantea* shells.


CNM
Length (mm)
Age (years)
85
75
65
55
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15
0
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4
6
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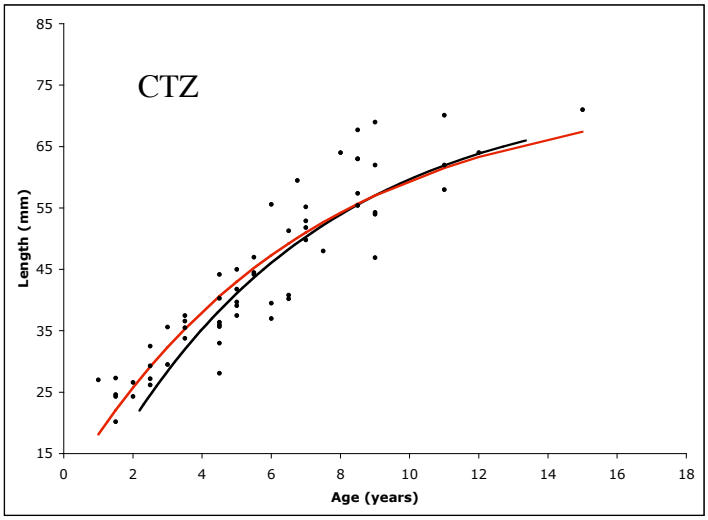
12
14
16
18


CTZ
Length (mm)
Age (years)
85
75
65
55
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35
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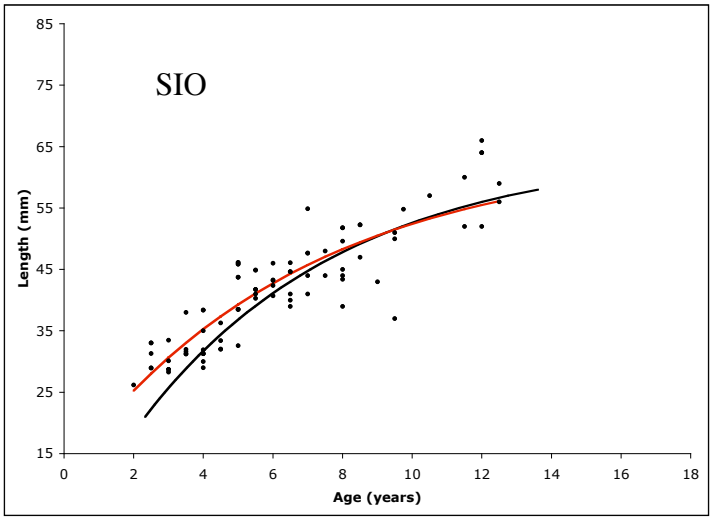
SIO
Length (mm)
Age (years)
85
75
65
55
45
35
25
15
0
2
4
6
8
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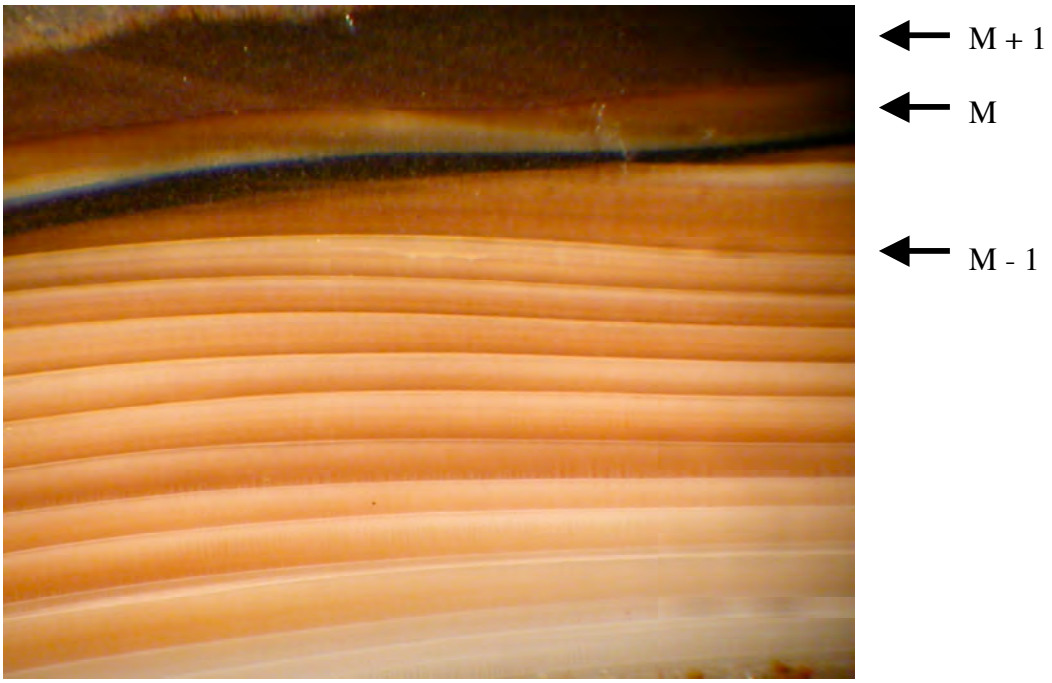
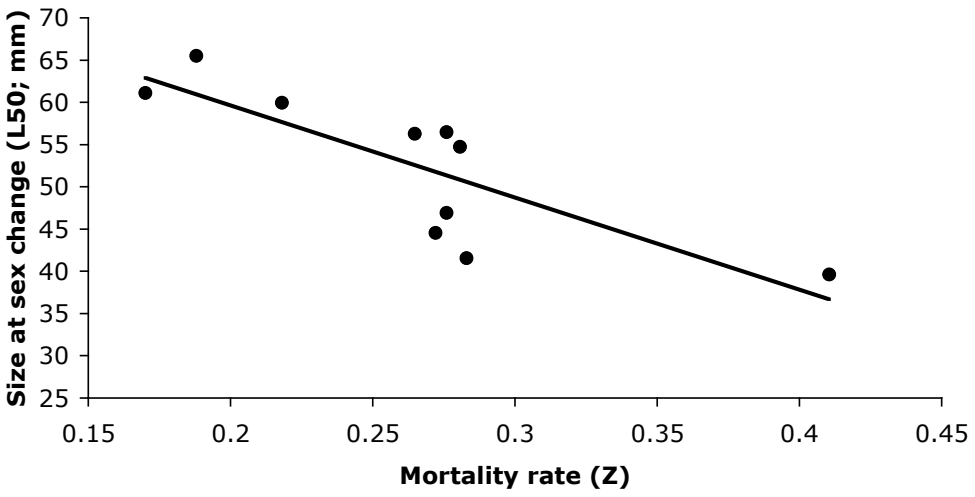
12
14
16
18



SI Figure 3-3. A cross-section view of an *L. gigantea* shell. Three distinct shell layers can be seen in this picture (M+1; M; and M-1). The M-1 layer of the shell contains easily identifiable growth increments that can be used to age individuals. Approximately 2 increments equals 1 year of growth.



SI Figure 3-4. A plot of size at sex change (L_{50}) versus total instantaneous mortality rate (Z) for each sampled *L. gigantea* population. The negative correlation indicates that populations with the highest mortality rates change sex at smaller sizes.

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CHAPTER 4
Genetic structure of the Owl limpet, *Lottia gigantea*

Introduction

The degree to which local populations of fish and invertebrate species are interconnected is a topic of great interest in marine biology (Grosberg and Cunningham 2001). Many marine populations are thought to be demographically open, with the addition of new individuals being largely dependent upon the influx of larvae from the plankton (Caley et al. 1996). Direct measurements of population connectivity are difficult to make (but see (Almany et al. 2007). As such, genetic methods are increasingly being used to indirectly measure connectivity and gene flow between local marine populations (Hellberg et al. 2002). Microsatellites are particularly useful genetic markers for such studies because they have the potential to provide modern estimates of migration rates and population connectivity that are generally not possible with markers with slower mutation rates, such as mtDNA (Selkoe and Toonen 2006).

However, studies of genetic connectivity among fish and invertebrate populations using microsatellites along the coast of California are rare. In fact, only one such study exists for an invertebrate species (Gruenthal et al. 2007). The harvested red abalone (*Haliotis rufescens*) was found to have no significant genetic divergence among California populations using microsatellites (Gruenthal et al. 2007), suggesting high levels of gene flow (but see AFLP measurements in Gruenthal et al. 2007). The lack of similar studies is surprising given the high diversity and ecological importance of invertebrate species along the California coast. In addition, such studies could potentially enhance our understanding of migration rates across possible phylogeographic

boundaries, such as Pt. Concepcion (Burton 1998).

In this study, we examine genetic structure using five polymorphic microsatellite loci across a large portion of the geographic range of a common California rocky intertidal gastropod species, *Lottia gigantea*. In particular, our aim is to examine if there are any significant geographic barriers to gene flow in this abundant and ecologically important member of the rocky intertidal of California (Lindberg et al. 1998; Kido and Murray 2003; Roy et al. 2003; Sagarin et al. 2007).

Lottia gigantea is a long-lived (upwards of 20 years; Fenberg and Roy in prep) broadcast spawning species with planktonic larvae. Reproductive individuals undergo a single spawning event in the winter months, typically in January or February (Daly 1975). Laboratory research on other northeastern Pacific *Lottia* species (*L. digitalis* and *L. asmi*) indicate that metamorphic competence occurs at 5.25-5.5 days after fertilization (at 13ºC), suggesting a relatively short pelagic larval phase for this genus (Kay and Emlet 2002). Even with a short pelagic phase, larvae entrained within the north-flowing coastal Davidson current during the winter months could potentially be transported long distances, upwards of 350 km (Glickman 1999; Gruenthal et al. 2007). Therefore, the potential for high rates of dispersal between local *L. gigantea* populations is substantial. However, other physical and biological traits may favor the local retention of larvae. For example, natural rocky intertidal habitat within the middle portion of the geographic range of *L. gigantea,* where they are most common (southern California), is often separated by long stretches of unsuitable sandy habitat. In addition, the potential for

predation and starvation of lecithotrophic *L. gigantea* larvae increases the longer they stay within the plankton.

Lottia gigantea individuals are size-selectively harvested for food by humans, which has resulted in significant reductions in body size at most California mainland populations (Pombo and Escofet 1996; Lindberg et al. 1998; Roy et al. 2003; Sagarin et al. 2007). The loss of large individuals can have cascading effects on the demography and life history of this species, as well as on the intertidal community as a whole (Fenberg and Roy in prep). Recent work shows that exploited local populations are significantly reduced in terms of both biomass and total reproductive output compared to protected areas (Fenberg and Roy in prep). Thus, harvesting may affect rates of gene flow by lowering total reproductive output. Considering that *L. gigantea* is a species of conservation concern, our genetic study also has the potential to help inform and implement sound management strategies.

Materials and methods

Sample sites and DNA extraction

We collected *L. gigantea* individuals between 2004 and 2006 at eight rocky intertidal field sites spanning most of the California mainland portion of its geographic range, from 32.66ºN to 38.18ºN (Figure 4-1). *Lottia gigantea* are rare north of San Francisco CA, therefore our sample sizes at Bolinas (37.89ºN) and Pt. Reyes (38.18ºN)

were reduced in order to limit damage to these populations. We stored individuals in 95% ethanol and extracted DNA using a QIAmp DNA minikit (Qiagen).

Genotyping and statistical tests

We used the *L. gigantea* genome (JGI) to screen for tri-nucleotide microsatellite markers with eight or more repeat units. In all, we genotyped six polymorphic markers for individuals from each of our sampled field sites. PCR amplification was performed using flourescently labelled primers (table 4-1) with an annealing temperature of 50ºC. Product sizes were visualized with an automated sequencer (ABI 3100). An internal size standard (400 HD ROX, Applied Biosystems) allowed for accurate sizing and the electropherograms were analyzed using GENEMAPPER software (Applied Biosystems). Alleles were scored as the PCR product size and converted to repeat number by subtracting the flanking regions. We calculated the number of alleles and the expected and observed heterozygosity values across field sites for each locus (table 4-2).

Samples were tested for significant deviations from Hardy-Weinberg equilibrium (HWE) across sites (multi-locus) and by locus (multi-site). Global and pairwise exact tests of genotypic differentiation were performed. In addition, we tested for evidence of linkage disequilibrium across all pairwise comparisons of loci. We performed the above statistical tests using the program GENEPOP v3.4 (Raymond and Rousset 1995; Raymond and Rousset 2003) with the following Markov chain parameters: 10,000 demorization steps, 1,000 batches, 10,000 permutations per batch (Gruenthal et al. 2007).

We also estimated F_{st} and the analogue P_{st} between all pairwise field site possibilities using GENEPOP (P_{st} takes into account allele size assuming a stepwise mutation model (Michalakis and Excoffier 1996; Rousset 1996). Levels of significance were based on sequential Bonferroni corrections at the $\alpha = 0.05$ level.

Clustering analysis

We used the program STRUCTURE (Pritchard et al. 2000) to further examine levels of genetic population structure across all sampled localities. STRUCTURE assigns individuals to subpopulations (k) independent of sampling sites and uses a Bayesian clustering method to assign individuals with similar multilocus genotypes to probable subpopulations of origin. We chose the admixture and uncorrelated allele frequency model and performed ten runs for each k (1-10) with a burnin of 30,000 steps followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations to estimate the mean and variance of log likelihoods and posterior probabilities of the number of assumed populations. We did not provide the program with a priori information about the population of origin for each individual. The best estimate of k that maximized the posterior probability of the data was determined by averaging the maximum likelihood scores for each k value. STRUCTURE also provides an estimate of the probability of the mean proportion of membership (q) for each individual within the assigned subpopulation. Individuals with q values = 0.90 or greater are considered to be confidently placed into their subpopulation of origin (Pritchard et al. 2000).

Results

Genetic structure

We found no evidence of linkage disequilibrium between any of the loci across all populations after sequential Bonferroni corrections (at the α=.05 level). In addition, we found no evidence of deviation from HWE across sites (multi-locus test) as indicated by the non-significant P-values for heterozygote deficit in table 4-2. However, we did find evidence for significant deviation from HWE for the *Lg6* locus (multi-site test; p=0.0005) after sequential Bonferroni correction. This may be due to any number of factors, including null-alleles, non-random mating, inbreeding or selection (Lowe et al. 2004). Although its inclusion did not significantly alter any of our results, we chose to exclude *Lg6* for the remainder of our analyses given the high probability that it is not a neutral marker. See table 4-2 for basic information on sample sizes (N), expected and observed heterozygosities (H_o and H_e respectively) and the number of alleles (A) observed for each locus across sampled field sites.

Analysis of all loci combined revealed no significant global differentiation (p=0.284). In addition, pairwise site comparisons revealed no significant patterns of genetic structure through analysis of genotypic differentiation and from the calculation of F-statistics (F_{st} and P_{st}) after sequential Bonferroni corrections (table 4-2). Geographic differences are only qualitatively apparent between the pairwise comparison of sites sampled at the latitudinal extremes of our study area (Cabrillo National Monument -

32.66ºN and Pt. Reyes National Seashore - 38.18ºN) as revealed by the relatively high Fst value (0.0221) and low p-value for genotypic differentiation (P=0.002) between these two sites (table 4-3). However, neither value remained significant after sequential Bonferroni corrections.

A lack of genetic population structure is further supported from our clustering analysis using the program STRUCTURE. Regardless of the assumed k, the proportion of membership (q) is equally partitioned into each subpopulation for all individuals. For example, at k = 2, the proportion of membership is equal to 0.50, at k = 1, the proportion of membership is 1.0 (Figure 4-2). Individuals with q values greater than 0.90 are considered to be confidently placed into their subpopulation of origin (Pritchard et al. 2000), therefore, our results indicate that genotyped individuals represent part of a single interbreeding population (k=1).

Discussion

We cannot reject the hypothesis of genetic homogeneity for the California mainland portion of the geographic range of *L. gigantea*. Such a result is not surprising given the potential for high rates of larval dispersal between populations (see above). In fact, numerous studies (using different genetic markers) have shown that little genetic structure exists along the California coast for invertebrate species with planktonic larval dispersal (Dawson 2001; Wares and Castaneda 2005; Gruenthal et al. 2007; Kelly and Eernisse 2007; Lee and Boulding 2007). For example, an allozyme study spanning the

California coast has shown that limited genetic subdivision exists in a solitary coral species with planktonic larvae, but that a co-occurring coral species with low-dispersing larvae shows the opposite pattern among local populations (Hellberg 1996). These and other studies along the California coast suggest that few long-term geographic barriers to gene flow exist for invertebrate species with high dispersal capabilities. However, our results are only directly comparable to the only other study along the California coast that used microsatellites to infer genetic structure for an invertebrate species (Gruenthal et al. 2007).

Like the red abalone (*Haliotis rufescens*), *L. gigantea* is primarily a winter broadcast spawning gastropod (Daly 1975; Gruenthal et al. 2007). Both species are harvested and have similar geographic ranges (northern California to central Baja California, Mexico) with slightly overlapping habitats. As such, it is not surprising to find that both species show no evidence of genetic structure using the same number of microsatellite loci (5) and comparable sampling localities. However, Gruenthal et al. (2007) show that AFLP markers (amplified fragment length polymorphisms) begin to show some evidence of genetic subdivision for *H. rufescens* populations, suggesting the need to include other markers for future studies.

However, considering that only a few migrants per generation can result in undetectable levels of differentiation (Taylor and Dizon 1996; Waples 1998), it is possible that our genetic methods are not capable at describing patterns of larval exchange in an ecologically meaningful context. This is particularly relevant for long-

lived species such as *L. gigantea* and abalone where chance recruitment events can have lasting effects on a local population (Gruenthal et al. 2007). Understanding the genetic structure of such species can be enhanced by sampling specific age cohorts and recruitment events (Hellberg et al. 2002). Unfortunately, our sampled individuals are time-averaged across multiple age-classes, making the detection of genetic differentiation more difficult. In addition, the average expected heterozygosities across all genotyped loci for our study (83%) are considered to be relatively high, which can potentially lead to dampened estimates of F_{st} and a reduced ability to detect structure (Selkoe and Toonen 2006).

Our results suggest that *L. gigantea* is a panmictic species along the mainland coast of California. From a conservation perspective, however, it is important to not exclusively rely on genetic data to make decisions on where to place management effort (Taylor and Dizon 1996). For example, certain portions of the range of a species (i.e. near range end-points) may be more naturally susceptible to population crashes and local extinction due to factors such as demographic stochasticity, physical factors, and historical effects (Holt and Keitt 2000; Guo et al. 2005; Holt et al. 2005), which would not necessarily be noticeable genetically. In fact, we have shown that census population sizes are significantly smaller at latitudes higher than Monterey Bay (>36.6ºN) compared with the middle portion of the range of *L. gigantea* (Fenberg in prep). In addition, juveniles (<25mm) are rare to absent at these higher latitudes, suggesting that the northern end of the geographic range of *L. gigantea* is demographically unstable. In fact, museum collections in combination with modern field surveys show that the northern

range limit of this species has contracted by ~2º of latitude over the last few decades (Fenberg in prep), suggesting that populations at higher latitudes are historically prone to localized extinction. Therefore, human impacts (i.e. harvest pressure) can have more pronounced effects and lead to a higher probability of localized extinction at higher latitudes.

Even in the absence of detectable genetic structure, these observations suggest that the northern range of *L. gigantea* (>36.6ºN) is demographically unstable and should therefore receive appropriate management efforts. Future studies will help to further explore the geographical relationship between demography and genetics and how they relate to inform management policy of this ecologically important species.

Table 4-1. Primers sequences and product sizes for 6 *L. gigantea* microsatellite loci.

Locus	left primer	right primer	~Size(bp)
Lg1 $(ATC)_n$	tgttcttggcatcatcaaactt	gcatcacaaaggtgcaaaga	263
Lg2 $(AAC)_n$	ttacaaccgaacagctcagg	gttggtgctgttgttgatgg	354
Lg3 $(AAC)_n$	caaagcgctagccctaaaac	ctgcggctgatttcttcttc	299
Lg4 $(TGA)_n$	aacatgaatgatttaggggaag	gctgtctttgtttttaaccgtgt	212
Lg5 $(AAC)_n$	gccgatattggttgattagaca	tatgctggttgttgcattgg	223
Lg6 $(GTT)_n$	acgacacggcatgtgtctta	gggatttagcgttttgcgta	259

Table 4-2. Descriptive statistics for 6 *L. gigantea* microsatellite loci across eight sampled field sites. Given are the number of samples (N), the observed (H_o) and expected heterozygosities (H_e) and the number of alleles (A) found per loci and locality. No significant evidence of heterozygote deficit was found for a multi-locus test across localities. However, *Lg6* was found to significantly deviate from HWE using an exact test for each loci (multi-locality; see text for details).

	Lg1(ATC)				*Lg2*(AAC)$_n$				*Lg3*(AAC)$_n$				*Lg4*(TGA)$_n$				*Lg5*(AAC)$_n$				*Lg6*(GTT)$_n$				Het. def
Locality(ºN)	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	N	H_e	H_o	A	P (SE)
Pt. Reyes(38.18)	14	.83	.79	7	13	.50	.54	4	15	.86	.93	11	15	.90	1.0	9	14	.91	1.0	12	15	.89	.87	12	0.89(0.00)
Bolinas(37.89)	18	.83	.78	8	18	.54	.55	7	18	.91	.94	12	18	.92	.89	11	16	.94	.94	13	17	.92	.82	14	0.13(0.00)
Pebble Beach(36.56)	28	.75	.71	8	25	.68	.68	7	27	.90	.88	13	27	.91	.96	12	28	.90	.82	14	24	.91	.88	14	0.11(0.00)
Ken Norris(35.52)	19	.69	.63	7	19	.62	.79	6	18	.88	.94	14	18	.93	.89	12	19	.93	.89	14	19	.90	.84	14	0.11(0.00)
Vandenberg(34.73)	23	.85	.83	12	25	.56	.56	9	24	.89	.83	14	22	.91	.95	11	24	.91	.83	15	25	.91	.84	15	0.22(0.00)
Palos Verdes(33.71)	35	.74	.77	10	31	.68	.68	8	31	.91	.94	17	37	.90	.95	14	46	.89	.93	14	38	.92	.89	15	0.85(0.00)
La Jolla(32.87)	35	.81	.80	11	25	.68	.80	8	32	.91	.91	17	35	.93	1.0	15	36	.92	.97	14	32	.92	.84	15	0.84(0.00)
Cabrillo N.M.(32.66)	28	.76	.82	10	28	.80	.75	9	26	.92	.96	15	28	.91	.79	13	28	.88	.86	13	22	.91	.91	14	0.16(0.00)
Sum	200			12	184			10	191			22	191			22	211			19	192			20	

Table 4-3. Global and pairwise comparisons of genotypic differentiation and F-statistics (F_{st} and P_{st}). No significant P-values existed after sequential Bonferroni correction at the $\alpha = 0.05$ level.

Comparison	Genotypic differentiation P-value	F-statistics Fst	Pst
Global	**0.284**	**0.002**	**-0.003**
BOL & PR	0.175	-0.001	-0.007
PBL & PR	0.455	-0.004	-0.001
PBL & BOL	0.747	-0.004	0.005
KN & PR	0.133	0.006	-0.003
KN & BOL	0.363	0.003	0.001
KN & PBL	0.325	-0.005	-0.009
VBG & PR	0.610	-0.004	-0.001
VBG & BOL	0.535	-0.005	-0.010
VBG & PBL	0.710	-0.002	-0.013
VBG & KN	0.310	0.000	-0.022
PV & PR	0.147	0.012	0.024
PV & BOL	0.418	0.001	0.032
PV & PBL	0.586	-0.004	0.005
PV & KN	0.506	0.000	-0.015
PV & VBG	0.910	0.000	-0.004
LJ & PR	0.092	0.008	-0.004
LJ & BOL	0.814	-0.004	-0.012
LJ & PBL	0.328	-0.001	-0.009
LJ & KN	0.667	-0.001	-0.008
LJ & VBG	0.648	0.001	-0.014
LJ & PV	0.939	0.000	0.013
CNM & PR	0.002	0.022	-0.005
CNM & BOL	0.154	0.012	0.002
CNM & PBL	0.215	0.002	-0.002
CNM & KN	0.074	0.009	-0.019
CNM & VBG	0.094	0.013	-0.017
CNM & PV	0.233	0.004	-0.005
CNM & LJ	0.350	0.005	0.002

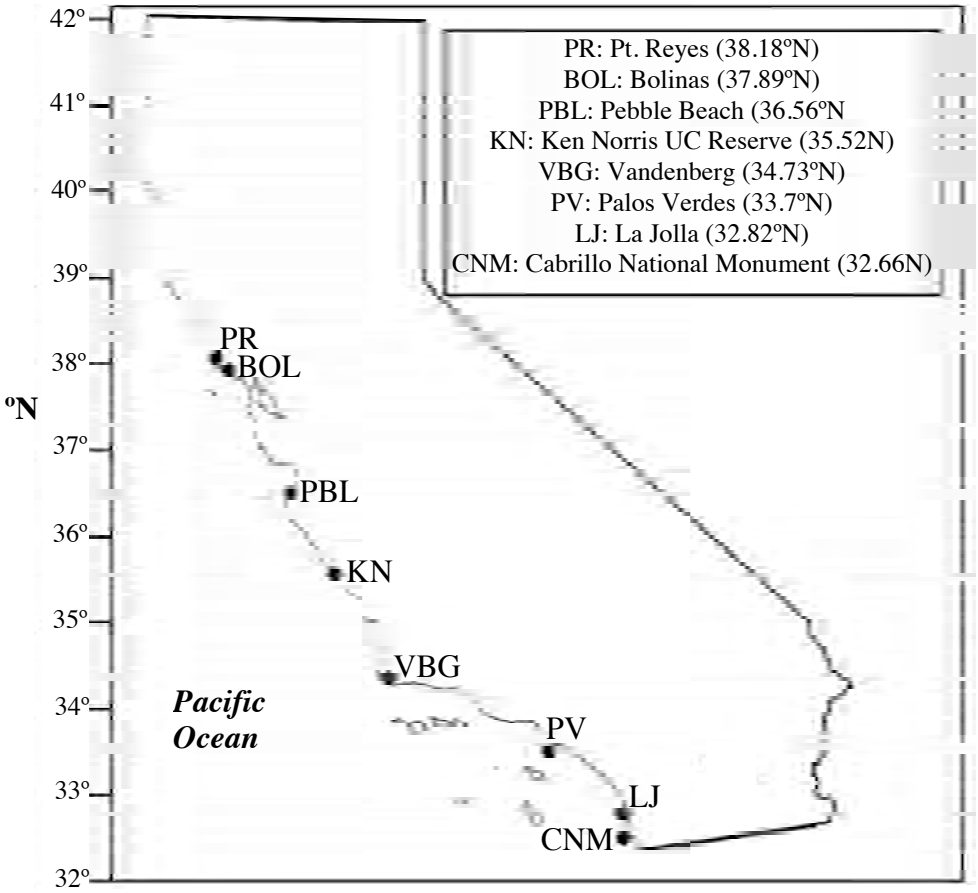
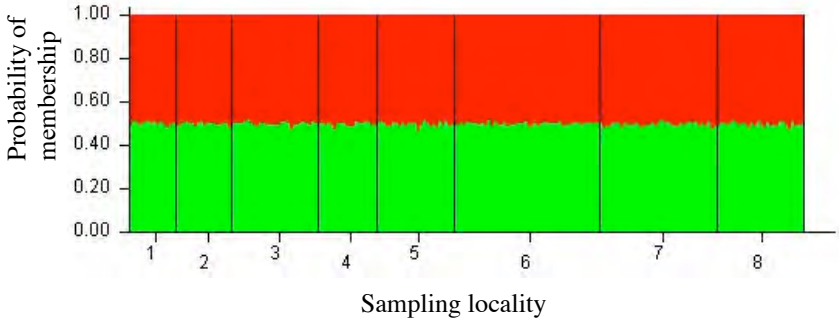


Figure 4-1. Map of California collections sites for *L. gigantea*.

A.



B.

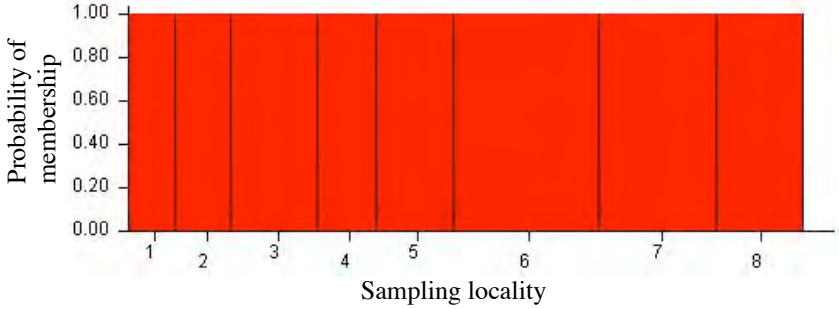


Figure 4-2. STRUCTURE bar plots of the proportion of membership (q) for individuals from sampled localities for assumed subpopulations (A). k=2 and (B). k=1. The proportion of membership is equally partitioned into each subpopulation (k=1-10) for all individuals, with a k=1 having the highest proportion of membership (q = 1.0). The numbered sampled localities (1-8) are arranged by latitude as shown in table 2.

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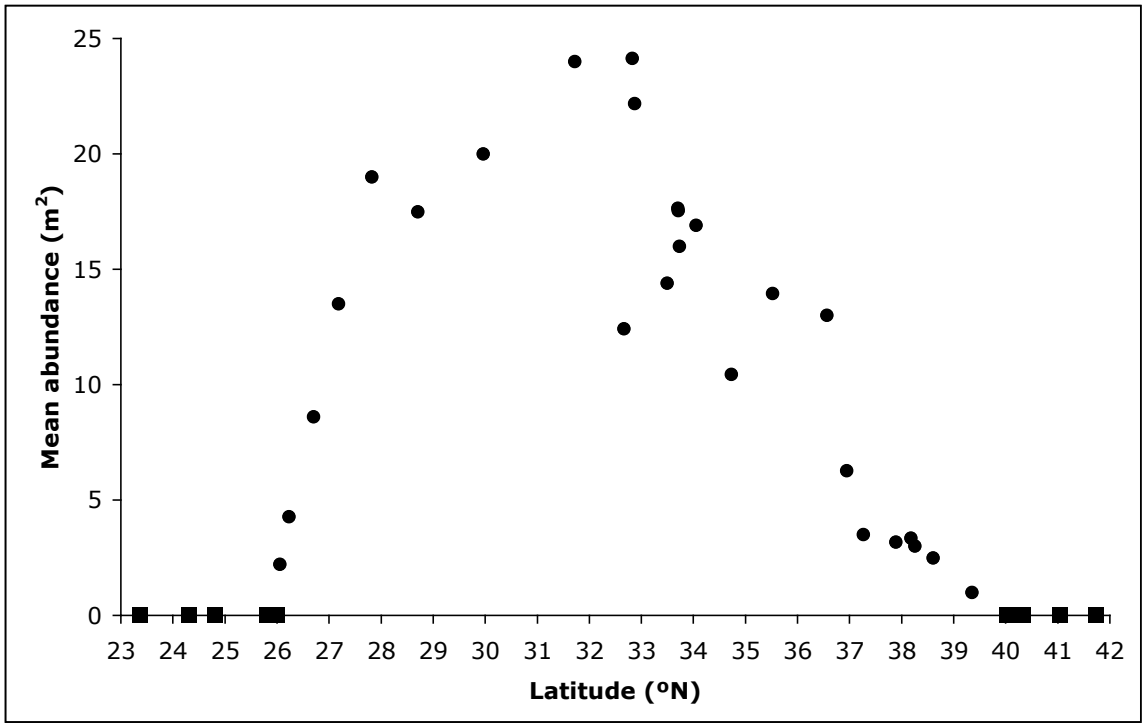
Appendix



Appendix Figure 1. The mean abundance per m^2 of sampled *L. gigantea* populations across its entire geographic range, from 26.05ºN to 39.35ºN. The black squares represent field sites that were extensively searched, but *L. gigantea* was not found. The northernmost black square represents the historical northern range limit of *L. gigantea* at Crescent City, CA (41.74ºN).