Food web impacts of geoduck clam aquaculture practices in Puget Sound, Washington

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PREFACE

Subsequent chapters of the thesis are formatted with the intent of eventual submission for publication in the appropriate peer-reviewed technical literature. Much of the introductory material for both chapters is contained within the following prologue, parts of which are repeated in the introductions of each chapter to facilitate fully informative stand-alone status. Sample collection for both chapters occurred concurrently, resulting in additional repetition between chapters in some of the methods, figures and tables.

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PROLOGUE

Decades of research have demonstrated the critical role that ecological disturbance plays in driving community dynamics (Grassle and Sanders 1973, Connell 1978, Sousa 1984, Sousa 2001). Disturbance can impact any level of ecosystem function (Pickett and White 1985), including trophic relationships, habitat use, reproduction, recruitment, growth and competition (Stouder and McMullin 2006). Factors that drive ecosystem and population level response to disturbance events include resistance, resilience, species diversity and richness, and the frequency, intensity, duration, size, predictability and chemical characteristics of the event itself (Paine et al. 1998, Peterson et al. 1998, Hughes et al. 2007). Many organisms are well adapted to certain types of perturbation, while others may experience highly deleterious effects. Disturbance can permanently change community structure or cause only temporary impacts leading to eventual recovery (Peterson et al. 1998). The extent and effects of disturbance events are highly varied, and it is not accurate to perceive perturbations as generally positive or negative.

Disturbances can be broadly classified as “pulse” (discrete, short) or “press” (chronic, longer-lasting) events (Glasby and Underwood 1996) and may be natural or anthropogenic in origin. Natural disturbance events, such as storms or fire, can have immediate negative impacts on certain species but are often crucial to overall maintenance of an ecosystem. For example, wild fires temporarily reduce the density of many organisms, but the ash left behind serves to replenish valuable nutrients in the ecosystem (Sousa 1984). Anthropogenic disturbances in aquatic environments, such as fishing and aquaculture, can imitate the natural disturbance regime of a particular habitat type, but repeated events may surpass the ability of an ecosystem to recover and retain its reference state (Sousa 1984, Paine et al. 1998).

Aquaculture operations are a frequent and prominent cause of anthropogenic disturbance in marine and estuarine communities. As human populations and their reliance on marine-derived protein sources continue to increase (Dumbauld et al. 2009), aquaculture is seen as a means for contributing to the fulfillment of the growing global demand for seafood (Costa-Pierce 2002). Worldwide, aquaculture of bivalve shellfish is growing. In the United States alone, shellfish culture generated $323 million in annual revenue and represented 35% of the total aquaculture industry in 2008 (FAO 2011). The growth of aquaculture activities has increased concerns about ecological impacts and accentuated the need for environmentally sensitive aquaculture management practices. The U.S. Commission on Ocean Policy (USCOP) and the National Oceanic and Atmospheric Administration (NOAA) recently prioritized research on sustainability and environmentally-sound regulation of bivalve aquaculture (USCOP 2004, NOAA 2006). Research on bivalve aquaculture disturbance has shown changes to nutrient and water cycling (e.g. Asmus and Asmus 1991, Giles and Pilditch 2006), sedimentation (e.g. Hayakawa et al. 2001, Hargrave et al. 2008) and biodiversity (e.g. Dealteris et al. 2004, Powers et al. 2007). There are several ways in which aquaculture can bring about changes to an ecosystem, including: 1) impacts from structural additions or “gear” used in the farming process, including the cultured organisms themselves; 2) influences from farmed organisms as they feed and excrete waste and 3) disturbance from harvest events (Dumbauld et al. 2009).

Successful regulation of aquaculture operations is dependent on ecologically oriented research on the effects of disturbances associated with culture activities and the creation of management plans that sustain yields while minimizing environmental impacts. In commercial aquaculture operations, physical structures such as nets, racks and bags are often used extensively and at high densities to contain and protect farmed organisms. Placement of structures constitutes a press disturbance that may affect multiple ecosystem processes for a number of years. Conversely, disturbance due to harvest of cultured species is typically a pulse event since it is short-lived in most cases (Dumbauld et al. 2009).

The ecological impacts of aquaculture operations depend on a variety of factors, including effects from within farms, such as the scope, size and location of cultured areas and landscape-scale effects, such as the density of operations throughout a region (Simenstad and Fresh 1995, Dumbauld et al. 2009). A variety of studies have researched the effects of bivalve aquaculture on the diversity and abundance of benthic and epibenthic invertebrates and mobile predators. The results are highly varied and range from no discernible impact to significant effects across multiple trophic levels. In some cases, the addition of artificial structure and cultured organisms can increase the diversity and abundance of meiofauna and macrofauna compared to unstructured sites (Dealteris et al. 2004, Powers et al. 2007, Erbland and Ozbay 2008). Algal growth on shellfish aquaculture gear may attract mobile invertebrates and fishes, resulting in greater abundance compared to uncultured, sandy-bottom areas (Powers et al. 2007). Moreover, mobile species may be drawn to structured sites to forage or to take refuge from predators (Guttierez et al. 2003). For example, natural and enhanced oyster reefs in South Carolina supported higher nekton abundance and greater species richness compared to soft-bottom reference sites (Kingsley-Smith et al. 2012). Attraction of foraging mobile predators to structured areas may result in reduced prey abundance (Hixon and Beets 1993). On the other hand, mobile species may be unable to reach smaller prey sheltered by structures, resulting in a potential increase in abundance of some prey species (Dealteris et al. 2004).

Depending on the site preferences of mobile organisms, changes in physical structure resulting from bivalve aquaculture may decrease space availability and alter movement patterns of predators away from farmed regions. Ribeiro et al. (2007) found that nearshore mussel aquaculture operations reduced foraging habitat for dolphins in Chile. Other studies noted no difference in the diversity and abundance of mobile species, such as fishes and crabs, between habitat types (e.g. Dumbauld et al. 2005, Hosack et al. 2006). One level of a food web may be impacted, while others are not. For example, Hosack et al. (2006) found distinct differences in benthic and epibenthic meiofauna between structured and unstructured areas in Willapa Bay, Washington, but no difference in mobile predator use of the same habitats. Within a trophic level some organisms can benefit from artificial structures, while others are impacted negatively or not at all. Such species-dependent results were found in several studies of infauna (e.g. Beadman et al. 2004, Murray et al. 2007) and nekton (e.g. Pregnall 1993, Rumrill and Poulton 2004). It seems likely that the ecological impacts of bivalve aquaculture gear will vary greatly depending on the particular farming circumstances, such as the species being cultured and the type and extent of gear used. The diverse outcomes of previous studies on bivalve aquaculture accentuate the need to research the impacts of disparate operations on local and regional scales in order to identify considerations that are applicable to each management planning effort.

Aquaculture of the Pacific geoduck clam (*Panopea generosa* Gould, 1850; henceforth geoduck) is a growing and valuable industry in Washington State and British Columbia and utilizes unique structures in the farming process. Geoducks are large, long-lived, burrowing clams found in soft intertidal and subtidal substrata from California to Alaska (Anderson 1971, Shaul and Goodwin 1982, Coan et al. 2000). In Washington State alone, geoduck farming and wild harvests generated an estimated $67 million in gross revenue in 2011 (NOAA 2013). In addition to generating jobs and significant revenue, geoduck harvests provide international trade opportunities, as many clams are exported overseas, primarily to Asia (GSGislason & Associates 2012).

Geoduck aquaculture involves high-density placement of structures to protect juvenile geoduck “seeds” from environmental stressors and predators such as crabs and birds (Figure 1.1). Seed clams are grown in hatcheries to approximately 5-10 mm shell length and outplanted in high-density to farmed areas. Polyvinylchloride (PVC) tubes are pushed partially into sandy tideflats of the intertidal zone and planted with up to four immature geoducks. Tubes are approximately 25-30 cm long and 15 cm wide. In general, 5-10 cm of each tube is exposed above the substratum. Tube fields are covered with large nets and all structures are left in place for one to two years, depending on geoduck growth rates. Alternatively and less commonly, a cap net secured to each tube with a rubber band may be used in place of larger nets that cover an array of tubes. Another alternative method uses mesh tubes instead of PVC tubes. After the nets and tubes are removed, the geoducks are left to grow for several more years until they reach market size of approximately one kilogram (GSGislason & Associates 2012). The clams are harvested five to seven years after planting. Harvest takes place with a hose, using a high volume of seawater at three atmospheres of pressure, which is inserted into the substrate, alongside of the geoduck siphons. Liquefaction of the sediment allows for rapid removal of the clams by hand, bypassing the need for labor-intensive removal of large sediment volumes by shoveling to gain access to clams. The farming cycle may be renewed, depending on the realized productivity of the specific site and prevailing permitting and economic circumstances.

While commercial geoduck aquaculture operations boost local economies and increase employment and international trade opportunities, currently there is limited knowledge regarding how geoduck farming alters marine environments (Straus et al. 2009). As operations rapidly expand, management agencies, conservation organizations and community groups have expressed concern regarding potential impacts on local habitat. Research on the impacts of other bivalve aquaculture operations is useful for developing hypotheses, however the novelty of geoduck farming practices necessitates particular investigation. As a result of the lack of information and mounting public concern as geoduck aquaculture expands in southern Puget Sound, the Washington State Legislature passed Second Substitute House Bill 2220 in 2007 (subsequently encoded in Washington State Law as RCW 43.21A.681). The bill mandated a series of scientific studies on shellfish aquaculture, including an assessment of the potential ecological effects of the nets and tubes used in geoduck aquaculture to protect young clams from predators.

Commercial geoduck aquaculture in Puget Sound is predominately concentrated in southern inlets with soft sandy to muddy substrata (Figure 1.2). Common macrobiota characteristic of the area include horse clam (*Tresus* spp.), cockle (*Clinocardium nutalli* [Conrad, 1837]), graceful crab (*Metacarcinus gracilis*, [Dana, 1852]), northern kelp crab (*Pugettia producta*, [Randall, 1840]), and Pacific sand dollar (*Dendraster excentricus* Eschscholtz, 1829). In addition, several species of commercial significance are known to inhabit the south sound region, including Dungeness crab (*Metacarcinus magister* [Dana, 1852]), salmonids (family Salmonidae), and English sole (*Parophrys vetulus* Girard, 1854). During the structured phase of the farming cycle, when nets and tubes are in place, there is an increased prevalence of green macroalgae (*Ulva* spp.) on the aquaculture nets and tubes, especially in the more productive summer months (personal observation).

Previous studies at geoduck aquaculture sites in South Puget Sound, Washington showed impacts to predator-prey dynamics and biodiversity. Brown and Thuesen (2011) found higher species richness at a geoduck culture site in the structured phase compared to a nearby unstructured control area. In another study, SCUBA surveys indicated that mobile predators common to the study region may exhibit foraging preference for structured or unstructured areas (McDonald et al. in press). For example, flatfishes such as English sole (*Parophrys vetulus* Girard, 1854) were more common in sandy bottom, unstructured areas, while crabs were observed more often at structured sites. Pacific staghorn sculpin (*Leptocottus armatus* Girard, 1854) were seen in high abundance at both habitat types, with preference for unstructured areas in some cases. An examination of the gut contents from staghorn sculpin captured at three cultured plots and their associated unstructured, reference plots in July 2009 showed significant differences between plots in the primary prey groups consumed (Smith and McDonald 2010).

Pacific staghorn sculpin (Scorpaeniformes, Cottidae) are opportunistic, benthic predators common throughout their geographical range from Baja California, Mexico to the Gulf of Alaska (Hart 1973). In parts of South Puget Sound where geoduck farming takes place, staghorn sculpin frequently occur in very high densities (up to one individual per square meter in some cases) and are often the most abundant fish in the community. Sculpin occupy various trophic levels within food webs and have a large mouth gape compared to their size (Armstrong et al. 1995). Staghorn sculpin are known to move with the tide into the intertidal zone to feed, and their diet consists of a wide variety of prey, including aquatic invertebrates, such as amphipods and crabs (Tasto 1975). Staghorn sculpin are also vital prey for a variety of birds and fishes (Fitch and Lavenberg 1975) and serve as an important link from lower to higher trophic levels. Because they are opportunistic feeders and occur in a variety of habitat types, staghorn sculpin are useful subjects for studying differences in the available energetic resources at disparate sites. Investigating differences in the consumption of predators such as staghorn sculpin at geoduck aquaculture sites is likely to inform an understanding of the effects of aquaculture operations on food web structure. Changes in population dynamics may cascade through food webs (Carpenter et al. 1985) and impact not only commercially important species, but the entire ecology of farmed regions, and accentuate the need for informed and sustainable aquaculture management practices.

CHAPTER 1

Diet and trophic ecology of Pacific staghorn sculpin (*Leptocottus armatus* Girard, 1854) at geoduck aquaculture farms and nearby reference areas

INTRODUCTION

Decades of research have demonstrated the critical role that ecological disturbance plays in driving community dynamics (Grassle and Sanders 1973, Connell 1978, Sousa 1984, Sousa 2001). Natural disturbance events, such as storms or fire, can have immediate negative impacts on certain species but are often crucial to overall maintenance of an ecosystem (Sousa 1984). Anthropogenic disturbances, such as fishing and aquaculture, can imitate the natural disturbance regime of a particular habitat type, but repeated events may surpass the ability of an ecosystem to recover and retain its reference state (Sousa 1984, Paine et al. 1998). Aquaculture operations are a frequent and prominent cause of anthropogenic disturbance in marine and estuarine communities. As human populations increasingly depend on aquatic food sources for protein and revenue, and populations of wild marine organisms become more depleted, aquaculture is on the rise worldwide (Costa-Pierce 2002). Successful regulation of aquaculture operations is dependent on ecologically oriented research on the effects of disturbances associated with culture activities and the creation of management plans that sustain yields while minimizing environmental impacts.

Aquaculture of Pacific geoduck clams (*Panopea generosa* Gould, 1850; henceforth geoducks) is a valuable industry in Washington State and British Columbia. In Washington State alone, geoduck farming and wild harvests generated an estimated $67 million in gross revenue in 2011 (NOAA 2013). While commercial aquaculture operations boost local economies and increase employment and international trade opportunities, currently there is limited knowledge regarding how geoduck farming alters marine environments (Straus et al. 2009). As operations rapidly expand, management agencies, conservation organizations and community groups have expressed concern regarding potential impacts on local habitat.

Geoduck clams are large, long-lived, burrowing clams found in soft intertidal and subtidal substrata from California to Alaska (Anderson 1971, Shaul and Goodwin 1982, Coan et al. 2000). Intertidal geoduck aquaculture involves high-density placement of nets and polyvinylchloride (PVC) tubes to protect outplanted juveniles from environmental stressors and predators such as crabs and birds (Figure 1.1). Predator-exclusion structures are removed once clams are of a certain size, generally one to two years after planting. The increased size and burrowed depth of clams at 1-2 years of age affords reduced vulnerability to natural predators. Research on bivalve aquaculture disturbance has shown changes to nutrient and water cycling (e.g. Asmus and Asmus 1991, Giles and Pilditch 2006), sedimentation (e.g. Hayakawa et al. 2001, Hargrave et al. 2008) and biodiversity (e.g. Dealteris et al. 2004, Powers et al. 2007). However, the context and scale of structures used in geoduck aquaculture have not been studied. In 2007 the Washington State Legislature passed Second Substitute House Bill 2220, mandating research on the effects of geoduck aquaculture practices, including evaluation of the ecological consequences of using predator-exclusion structures.

Disturbances from structure additions may cascade through food webs, negatively or positively impacting species at each trophic level. Research shows enormous variety in the impacts of structures on food web ecology. The addition of aquaculture structures and cultured organisms can increase the diversity and abundance of meiofauna and macrofauna compared to unstructured sites (Dealteris et al. 2004, Powers et al. 2007, Erbland and Ozbay 2008). Algal growth on shellfish aquaculture gear may attract mobile invertebrates and fishes, resulting in greater abundance compared to uncultured, sandy-bottom areas (Powers et al. 2007). Moreover, mobile species may be drawn to structured sites to forage or to take refuge from predators (Guttierez et al. 2003). On the other hand, aquaculture structures can have no effect on the diversity and abundance of mobile species (e.g. Dumbauld et al. 2005, Hosack et al. 2006) or may impact one level of a food web, while other levels remain undisturbed (e.g. Hosack et al. 2006). It is also possible that changes in physical structure may decrease space availability and alter movement patterns of some predators away from farmed regions (e.g. Ribeiro et al. 2007).

The presence of physical structure can also impact trophic dynamics. Some studies found that artificial and natural oyster reefs support higher trophic level predators than nearby soft-bottom habitats (Simonsen 2008, Quan et al. 2012). Oyster reef food webs in Lavaca Bay, Texas were more complex, with a greater number of links and predators of higher trophic position compared to marsh habitat (Wrast 2008). Such distinctions are ecologically significant, because food web complexity impacts resilience (May 1974) and resistance and thus, the ability of an ecosystem to withstand and recover from perturbation (Vallina and Le Quéré 2011). In the event of environmental disturbance, more complex food webs are generally associated with lower resilience due to competition between species (May 1974, McCann et al. 1998). However, increased interactions in complex systems are thought to sustain higher levels of biodiversity (Kokkoris et al. 1999). Within a population, trophic position is linked to the success and adaptability of an organism (de Ruiter et al. 2005) and therefore has direct implications for population health and conservation management.

Evaluations of food web interactions can provide important insights into understanding ecosystem complexity and the effects of disturbance. It seems likely that the ecological impacts of bivalve aquaculture gear will vary greatly depending on the particular farming circumstances, such as the species being cultured and the type and extent of gear used. The diverse outcomes of previous studies on bivalve aquaculture accentuate the need to research the impacts of disparate operations on local and regional scales in order to identify considerations that are applicable to each management planning effort.

Previous studies at geoduck aquaculture sites in South Puget Sound, Washington showed impacts to predator-prey dynamics and biodiversity. Brown and Thuesen (2011) found higher species richness at a geoduck culture site in the structured phase compared to a nearby unstructured control area. In another study, SCUBA surveys indicated that mobile predators common to the study region may exhibit foraging preference for structured or unstructured areas (McDonald et al. in press). For example, flatfishes such as English sole (*Parophrys vetulus* Girard, 1854) were more common in sandy bottom, unstructured areas, while crabs were observed more often at structured sites. Pacific staghorn sculpin (*Leptocottus armatus* Girard, 1854) were seen in high abundance at both habitat types, with preference for unstructured areas in some cases. An examination of the gut contents from staghorn sculpin captured at three cultured plots and their associated unstructured, reference plots in July 2009 showed significant differences between plots in the primary prey groups consumed (Smith and McDonald 2010).

Pacific staghorn sculpin (Scorpaeniformes, Cottidae) are opportunistic, benthic predators common throughout their geographical range from Baja California, Mexico to the Gulf of Alaska (Hart 1973). In parts of South Puget Sound where geoduck farming takes place, staghorn sculpin frequently occur in very high densities (up to one individual per square meter in some cases) and are often the most abundant fish in the community (personal observation). Staghorn sculpin are known to move with the tide into the intertidal zone to feed, and their diet consists of a wide variety of prey, including aquatic invertebrates, such as amphipods and crabs (Tasto 1975).

Staghorn sculpin are also vital prey for a variety of birds and fishes (Fitch and Lavenberg 1975) and serve as an important link from lower to higher trophic levels. Because they are opportunistic feeders and occur in a variety of habitat types, staghorn sculpin are useful subjects for studying differences in the available energetic resources at disparate sites. Investigating differences in the consumption of predators such as staghorn sculpin at geoduck aquaculture sites is likely to inform an understanding of the effects of aquaculture operations on food web structure.

While stomach content analysis (SCA) and direct observation of feeding are traditional approaches used to study predator diet, each come with limitations. Both categories of data provide only instantaneous measures of diet and are associated with significant biases. In stomach content analysis, soft-bodied prey may be underrepresented compared to hard-bodied organisms since they are likely to pass through the digestive system faster (Sheppard and Harwood 2005), be rendered unidentifiable more quickly by digestive processes, and leave fewer hard-part remains necessary for identification to lower taxonomic levels. Direct observation of feeding can be challenging, as seeing each prey item as it is consumed is generally not possible, especially if prey are not large enough for visual detection. In addition, fish foraging often involves quick and unpredictable movements that are difficult to observe, especially without using SCUBA or snorkeling methods. However, SCUBA and snorkeling include inherent limitations, such as low visibility under certain water conditions, and behavioral changes to fish and prey that may result from disturbing organisms. It is clear from the literature and from the studies noted above that many common food sources (e.g. amphipods and polychaetes) for staghorn sculpin cannot be identified to lower taxonomic levels without a dissecting microscope. Chemical examinations of diet, such as stable isotope analysis (SIA), compliment traditional methods, such as SCA, in elucidation of food web dynamics. While gut content data provide a detailed, instantaneous snapshot of what a consumer ingested, stable isotopes provide information about longer-term nutrient assimilation. Combining the two methods is a powerful approach to examining trophic relationships and foraging ecology (e.g. Kadye and Booth 2011, Polito et al. 2011).

In the past several decades, SIA has emerged as an extremely valuable tool for studying marine food web ecology (Fry 2006). Stable isotope analyses supply information about a consumer's trophic level and diet, and the primary producers of greatest significance to the base of food webs within which a consumer participates. The timeline over which stable isotopes reflect diet (half-life) varies by organism and the type of tissue tested (Madigan et al. 2012; see Methods for further detail). For example, in general, liver has a shorter half-life than blood and blood has faster turnover than muscle tissue (e.g. MacNeil et al. 2006). SIA takes advantage of predictable changes in specific "stable" (i.e., non-radioactive) isotopes of certain elements to track energy movement through food webs. Heavy isotopes of carbon and nitrogen (^{13}C and ^{15}N) and their more common light isotopes (^{12}C and ^{14}N) are compared as ratios (^{13}C/^{12}C and ^{15}N/^{14}N) and measured in target organisms. The change in isotopic ratio that occurs between trophic levels is called fractionation. Ratios of stable carbon isotopes reflect diagnostic characteristics of categories of primary producers (e.g. phytoplankton vs. benthic algae) that support particular food webs. Stable carbon isotopes generally do not fractionate, or may fractionate very little, as biomass moves through a food web, thus allowing for the identification of the principal source of primary production at the base of the food web to which the subject consumer is trophically connected (Peterson et al. 1985, Vander Zanden and Rasmussen 2001). Large differences in stable carbon isotopic signatures between organisms indicate that they have different food sources or that their food webs are based on different primary producers. For example, macroalgae are often more enriched in ^{13}C than phytoplankton, so organisms from food webs based on benthic algae are likely to have higher carbon signatures than those from plankton-based carbon sources (France 1995). However, the carbon signatures of primary producers vary greatly depending on factors such as latitude and temperature (Hemminga and Mateo 1996), so in some cases benthic food webs may be more depleted in ^{13}C than planktonic food webs (Jennings et al. 1997). Unlike stable carbon isotopes, stable nitrogen isotope ratios are known to vary by a consistent proportion as organic matter is passed from one trophic level to the next higher level and can thus be used to estimate the trophic position of a consumer and investigate trophic relationships in a food web (Peterson et al. 1985, Peterson and Fry 1987, Fry 1988).

Stable isotope analysis has a wide range of ecological applications and is a common tool for studying food web dynamics. For example, stable isotopes were used to show that the structures used in Pacific oyster (*Crassostrea gigas* Thunberg, 1973) aquaculture impact the trophic positions of polychaetes (Dubois et al. 2007) and to demonstrate differences in trophic levels of fishes between distinct, nearby habitats (Jennings et al. 1997, Deudero et al. 2004). Gray et al. (2004) used carbon stable isotopes to confirm the site fidelity of slimy sculpin (*Cottus cognatus* Richardson, 1836) to neighboring habitats with different environmental features in a Canadian agricultural region.

Differences in consumer trophic position and sources of carbon are important factors to consider when elucidating ecological changes initiated by disturbance events, such as the placement of gear used in aquaculture operations. Changes to food web dynamics may modify the composition of an ecological community, alter trophic regulation (e.g. the relative contributions of "bottom-up" and "top-down" trophic influences) and initiate trophic cascades

(Carpenter et al. 1985, Power 1992). Such significant changes to community structure warrant particular scientific investigation in order to effectively conserve and manage ecological resources.

Because of their high densities at farmed and unfarmed areas in the study region, staghorn sculpin were used as an indicator species to investigate the ecological impacts of the structured phase of geoduck aquaculture. The following questions were posed:

1. Do staghorn sculpin show fidelity to farmed and unfarmed habitats?
2. Do the diets of staghorn sculpin differ between the two habitat types?
3. Does geoduck aquaculture initiate changes in energy flow through a food web, using staghorn sculpins and their prey assemblages as a case study?

To address these questions, mark-recapture was employed to investigate habitat fidelity, and stable isotope and gut content analyses were used to assess differences in carbon sources, trophic position and diet at sites with and without geoduck aquaculture operations.

MATERIALS AND METHODS

Study Area and Design

All study sites are located in Case Inlet in southern Puget Sound, Washington State. Each site was leased by Taylor Shellfish Farms, Inc. (Shelton, WA) for the purpose of farming geoduck clams. The sites used were “Foss” in 2011 and Foss, “Manke” and “Rolfs” in 2012. Foss (N47°13.447’ W 122°49.126’) is located on the eastern shore of Case Inlet near Joemma Beach State Park. Manke (N47°12.104’ W 122°50.263’) and Rolfs (N47°14.329’ W 122°51.548’) are situated on the western shore of Case Inlet, on Harstine Island (Table 1.1, Figure 1.2). All sites consisted of a farmed/structured “cultured” plot and an associated unfarmed/unstructured “reference” plot, each with an area of 2500 m^2. Cultured plots occurred on active geoduck farms in the structured phase of the farming cycle, with cover nets and tubes in place for the duration of the study. Reference plots were areas without aquaculture and no history of geoduck aquaculture, located at least 100 meters from each cultured plot. Plot shape, tidal height and general resident species composition were similar between sites and plots prior to aquaculture operations. However, benthic assemblages at the sites were by no means identical, as noted in McDonald et al. (in press) and VanBlaricom et al. (in press). Both studies documented spatial and temporal variability in the intertidal benthic assemblages of Case Inlet.

Data Analysis

Data analysis was conducted in R (R Development Core Team, version 2.14.2) with the community ecology package, “vegan” and the model selection package, “AICcmodavq”. A p-value of 0.05 was considered significant in statistically-based contrasts.

Mark-Recapture

In order to investigate site fidelity, Pacific staghorn sculpin were collected monthly with a pole seine (length 8.7 m, height 1.7 m) during ebb tides in July-September 2011 and April-September 2012 at cultured and reference plots. Sculpin over 65 mm total length were tagged, measured to the nearest mm and weighed to the nearest 0.1 g before a recovery period and subsequent release back onto the plot of capture. Uniquely numbered T-bar anchor tags (product number FF-94 super short, Floy® Tag & Manufacturing, Inc., Seattle, Washington USA) were secured with fine fabric tagging guns just below the dorsal fin, into the muscle tissue between the spinal processes. Effectiveness of the tagging procedure (tag retention and mortality, see Results for further detail) was tested in the laboratory prior to beginning fieldwork. Tags and needles were sterilized with providone-iodine solution in the laboratory and with 70% ethanol in the field prior to tagging each fish. Before tagging, staghorn sculpin were anesthetized in a water bath with medical grade carbon dioxide from a compressed gas cylinder. Recaptured sculpin were weighed, measured and released to the plot of most recent capture. For each seining event, a random subset of the staghorn sculpin less than 65 mm (at least 50 individuals/plot, if available) was measured before release to the plot of capture.

Stomach Content Analysis

Stomach contents were analyzed monthly for at least eight non-empty staghorn sculpin caught on ebb tides at each site and plot from May through September, 2012. Because the guts of staghorn sculpin caught during flood tides in earlier studies were mostly empty (R. Smith and P.S. McDonald, unpublished data), the study assumed that sculpin moved in with the tide to feed at cultured or reference plots, remaining until the tide receded. Thus, sculpin gut contents were assumed to be from a feeding event that occurred at the location where the individual was caught, in the few hours immediately preceding capture (see also results section for supporting mark-recapture data).

A random subset of fish captured during seining events was euthanized in a buffered water bath with MS-222. Tissues were fixed in buffered 10% formalin and transferred to 70% isopropanol after 24-72 hours. Prior to stomach extraction, each fish was blotted dry, weighed and measured. Gut content analysis included weighing the full and empty stomach and identifying, enumerating and weighing the contents to the lowest possible taxonomic level. All weights were blotted dry wet weights to the nearest 0.0001 g.

Stomach content data were computed as the proportional weight contribution of each prey type to the total mass of stomach contents per month and habitat type (Table 1.2). Guts that were empty or contained only unidentifiable material were excluded from the dataset. Prey groups were created based on broad taxonomic group and frequency of occurrence, resulting in the following eight categories: amphipods excluding *Americorophium salmonis* (Stimpson, 1857) and *Monocorophium* spp. (AMPH), bivalves (BIV), the amphipod species *Americorophium salmonis* and *Monocorophium* spp. (CORO), crabs (CRAB), isopods (ISO), other crustaceans including ostracods, cumaceans and tanaidaceans (OCRUST), polychaetes (POLY), shrimp (SHRI) and other identifiable prey items not belonging to any of the previous eight groups (OTH).

Stomach Content Data Analysis

Stomach content data were pooled across sites and sculpin size for the following reasons: 1) all sites were located in close proximity to one another and have functionally similar habitat (see also above for description of sites); 2) there was little variation in the size of fish within each month; 3) the primary objectives of the study were to examine differences between farmed and unfarmed areas, as opposed to focusing on distinctions between sampling sites and 4) in some cases the sample sizes would be smaller than practical for the multivariate methods applied if the data were further separated.

Gut content data were arcsine square root transformed. Arcsine square root transformation is often used for proportion data to spread the higher and lower proportions and compress mid-level proportions (Zar 2010). Analysis of similarity (ANOSIM) was used to test for differences in gut content prey groups between months and cultured and reference plots. This non-parametric method tests for significance by ranking pairwise combinations of objects based on their similarities (Clarke 1993). ANOSIM was conducted on a matrix of Bray-Curtis dissimilarities (Bray and Curtis 1957) of the prey group proportions. A Monte Carlo permutation test with 999 iterations generated the test statistic (R) and a p-value. Values of R range from -1 to 1. Positive R values indicate differences among groups, while values of zero indicate no differences between groups (McCune et al. 2002). Negative R values suggest that the biggest differences are within groups instead of between groups (Clarke and Gorley 2001). Ecological importance is generally interpreted as follows: low to none when R is less than 0.2, moderate when R is between 0.2-0.45 and high when R is above 0.45 (Howe 2006).

Non-metric multidimensional scaling (NMDS: Kruskal and Wish 1978) was employed to explore visual representations of diet at cultured and reference plots. NMDS is an ordination technique that works complementary to ANOSIM and reduces the dimensionality of a dataset based on the rank order of distances between objects (Digby and Kempton 1987). It reflects no assumptions of linearity and can be applied to any dissimilarity matrix (McGarigal et al. 2000), which makes it an especially useful tool for investigating relationships in non-normal species abundance datasets (McCune et al. 2002). NMDS was conducted on a Bray-Curtis dissimilarity matrix of the arcsine square root transformed, diet proportion data. Iterations were run 1000 times to ensure convergence with minimal stress (an indication of the strength of correlation between the original distance measures and the distance measures in NMDS ordination space). Variable weights were calculated by performing a linear correlation of prey groups and NMDS axis scores. Statistical significance of prey groups was based on a permutation test. Significant prey groups were overlaid as vectors on the NMDS plots to facilitate interpretation of the position of each fish's stomach contents in ordination space.

Stable Isotope Sample Collection

Sediment cores (0.00196 m^2) and epibenthic pump samples (0.0196 m^2) were collected in June and August 2012 at all sites and both habitat types. Prior to sampling, each plot was converted to a Cartesian grid of 100x100 units in Microsoft Excel. A random number generator was used to select points for the infaunal cores. Selected locations were not sampled more than once during the project. Epibenthic invertebrates were collected on the ebb tide while water partially covered the plot. Infaunal cores were collected during low tide near the epifaunal sampling locations and from an area undisturbed by the epifaunal sampling process. A PVC tube (diameter 5 cm, length 15 cm) was used for core collection. Epifaunal samplers (Figure 1.3) were based on a design used by Toft and Cordell (2006). During sample collection, the base of the sampler (diameter 15.8 cm) surrounded an area of substrate and a hand pump drew the sample into a 250-micron mesh collection bag attached with a hose clamp to the pump outflow point. Water flowed through the bag, trapping organisms larger than the mesh size. Holes in the sampler base allowed water to enter the device. The holes were covered with 150-micron mesh so no additional epifauna could be sampled from an area other than the enclosed substrate. After 50-100 pumps, the sample bag was removed and secured in a jar and a clean bag was attached for the next sampling point.

In the field, infaunal and epifaunal samples were stored on ice immediately after collection. In the laboratory, samples were refrigerated until processing, which occurred within 72 hours of collection. Infaunal and epifaunal samples were sieved through a 500-micron mesh screen, sorted into sculpin prey groups, rinsed with deionized water and frozen at -20°C until processing for stable isotope analysis.

A random subset of staghorn sculpin captured during seining events was euthanized in a buffered water bath with MS-222 and preserved in the field on ice for SIA. Fish were stored in the laboratory at -20°C until processing.

Stable Isotope Processing and Analysis

All samples for SIA were freeze-dried, weighed in tin capsules to the nearest 0.001 mg and sent to the University of California Davis Stable Isotope Facility for analysis. Benthic and epibenthic invertebrates were generally weighed whole after freeze-drying. Some larger invertebrates (e.g. certain crabs and polychaetes) were ground into a fine powder with a mortar and pestle before weighing. For staghorn sculpin, muscle tissue was dissected from each fish, freeze-dried and ground into powder as noted above. Every effort was made to reduce cross-contamination of utensils and labware during the dissecting, grinding and weighing procedures. Stainless steel scalpels, forceps, spatulas, mortars and pestles, etc. were deep cleaned with methylene chloride between each batch of samples and rinsed with 70% ethanol between each sample. Gloves were also changed regularly.

At the stable isotope laboratory, samples were analyzed for stable carbon and nitrogen isotopes via isotope ratio mass spectrometry. The analytical procedure combusts samples and removes moisture and oxides. Nitrogen and carbon dioxide are separated in a gas chromatographic column before entering a mass spectrometric detector. Throughout analysis, samples are measured against standard reference materials. An isotope ratio is calculated for each sample relative to reference gases (Fry 2006).

Stable Isotope Data Analysis

Carbon ($\delta^{13}C$) and nitrogen stable isotope data ($\delta^{15}N$) were expressed as ratios (R) of the heavy isotope to the light isotope ($^{13}C/^{12}C$, $^{15}N/^{14}N$) and compared to an internationally recognized reference standard, where $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$. Units were expressed as parts per thousand or per mil (‰). The standards were Vienna Pee Dee belemnite limestone for carbon and atmospheric N_2 for nitrogen.

Carbon and nitrogen stable isotopes were analyzed for staghorn sculpin captured at all sites and both plot types for the months of May, July and September. Sculpin prey groups were pooled from infaunal and epifaunal samples and across sites and analyzed as sample mass allowed, resulting in data for cultured and reference plots in the months of April, June and August for the following groups: AMPH, CORO, CRAB, filter-feeding polychaetes (FPOLY), predatory polychaetes (PPOLY) and SHRI. Prey group data from April, June and August were matched with sculpin data from May, July and September, respectively. Isotopic turnover rate increases with trophic position and data on species-specific rates are generally lacking. A number of studies suggest that the half-life of carbon and nitrogen in the muscle tissue of small or fast-growing fish is two to six weeks (Zuanon et al. 2006, Guelinckx et al. 2007, Weidel et al. 2011), so it is not unreasonable to match prey group signatures from one month with sculpin signatures from the next.

An information-theoretic approach using Akaike's information criterion (AIC; Akaike 1973) was employed to analyze carbon and nitrogen stable isotope data from staghorn sculpin. Increasingly popular for analysis of ecological data, AIC holds several advantages over traditional hypothesis testing methods (Symonds and Moussalli 2011). Instead of using step-wise comparisons, AIC compares multiple models at once and ranks each model according to goodness of fit with an AIC value. The user can compare models and variables to one another by assessing differences in goodness of fit (Burnham and Anderson 2002, Garamszegi 2011). Akaike's information criterion does not use p-values or notations of significance. Instead, the focus is on the reliability or precision of each model.

For the sculpin stable isotope data, a list of candidate linear mixed effects models was created based on plot (P), month of sampling (M) and sculpin length (L) for the response variables of $\delta^{13}C$ and $\delta^{15}N$. Since the effect of site was minimized in the experimental design, site was included as a random effect on the intercept of each model. Plot, month and fish length were fixed effects. A modified version of AIC (AICc) that adjusts for small sample sizes was used: AICc = -2*ln(L) + 2*K + (2*K*(K+1))/(n-K-1), where L is the maximum likelihood estimate for the model, K equals the number of parameters including the intercept and n is the number of data points. Delta AICc (ΔAICc) is the difference between each candidate model and the model with the lowest AICc value. Models with ΔAICc values less than 2 are considered to be equivalent in quality of fit to the top model (Richards 2005). The strength of each candidate model (i) was compared using an Akaike weight (W_i) and an evidence ratio (ER; the weight of the best model divided by the weight of another candidate model). Akaike weights were calculated from ΔAICc values and represented the probability that a specific model was the model of best fit (Burnham and Anderson 2002).

After generating AICc values, the original list of candidate models was narrowed to generate a 95% confidence set of models with cumulative Akaike weight ≤ 0.95 (Symonds and Moussalli 2011). The resulting models were used to calculate the average weight, standard error and 95% confidence interval for each model parameter (plot, month, sculpin length and the interaction terms) to assess the effect of each variable on the response variable. Categorical parameters were compared to a base model such that cultured was compared to reference for plot and the months of July and September were compared to May. If zero was included in the confidence interval, the estimate was considered to be no different from zero and, thus, it was assumed that there was no effect of the variable tested (Mazerolle 2006).

Stable Isotope Mixing Model

The Bayesian stable isotope mixing model (SIMM) Stable Isotope Analysis in R (SIAR; Parnell et al. 2010) was used to explore contributions of each prey item in the diets of staghorn sculpin. Bayesian models are useful for interpreting stable isotope data in food web studies because they incorporate variability and uncertainty in the system by allowing for input of prior information, such as fractionation factors and data from SCA, in addition to the stable isotope values and variances for the consumer and each food item or "source" (Moore and Semmens 2008). The model corrects the data for isotopic fractionation and uses an algorithm to output probability distributions for each prey item. Unlike other Bayesian stable isotope mixing models, SIAR uses an error term to account for unknown sources of error (Parnell et al. 2010).

Sculpin stable isotope data from cultured and reference plots were pooled across sites and entered into SIAR for the months of May, July and September. Source group data for the months of April, June and August at both plots included AMPH, CORO, CRAB, POLY (FPOLY and PPOLY combined) and SHRI. Due to lack of sample mass, there were no data for reference plot shrimp in April or June, so the shrimp values from the cultured plot for the same months were used as a substitute. There was also not enough sample to conduct SIA on the remaining prey groups (BIV, ISO, OCRUST and OTH) isolated in the SCA, however the most common sculpin food sources from the guts are represented by AMPH, CORO, CRAB, POLY and SHRI (see Table 1.2 and Figure 1.4). Isopods are also an important group, but primarily only for one month-plot combination (June reference), which was not represented in the modeling period. Nonetheless, it is recognized that mixing models are only as useful as the data available and are limited by potential lack of information for all possible food sources (Polito et al. 2011). The unknown source contribution ("UNKN") was found by subtracting the sum of the medians of the five prey groups from one.

Fractionation factors (^{13}C: 0.8 ± 0.090 SD, ^{15}N: 3.4 ± 0.102 SD) were taken from the literature (Yokoyama et al. 2005) and mass-based proportions of the five prey groups from SCA were entered into SIAR for each plot and month. Stable isotope data for the two polychaete groups were combined for the mixing model because polychaetes in the sculpin guts were generally too digested to assign to the FPOLY or PPOLY group. The proportions of each prey group from SCA were recalculated to exclude the four groups listed above that lacked stable isotope data (Table 1.3).

Fitness Metrics Analyses

The following metrics analyzed from the gut content data were used to assess the overall fitness of sculpin at cultured and reference plots: 1) ratio of sculpin mass (total prior to gut removal) to the mass of the gut contents as a metric of gut fullness and 2) ratio of sculpin total length to sculpin mass as a metric of body condition. Analysis of variance (ANOVA) was used to test for significant differences between plots and months. To meet the assumptions of ANOVA, the data were log transformed prior to analysis.

To prevent a bias related to allometric scaling between plots in the ratio of sculpin length to sculpin mass, only fish within specified 20 mm length ranges were analyzed for each month. The aquaculture structures likely caused a sampling bias since sculpin at the cultured plots had more opportunity to escape the seine than at the reference plots. Smaller sculpin at the cultured plots could fit through the mesh of the aquaculture netting, allowing them use the nets and tubes as refuge, and fish of any size could escape if the seine was briefly snagged on the aquaculture structures. Only sculpin of lengths represented at both plot types were used in the data analysis.

Sculpin size ranges were based on total lengths of all fish captured and designated for each month: May (45-65 mm), June (60-80 mm), July (80-100 mm), August (95-115 mm) and September (105-125 mm). Tests between months were not conducted for this metric due to differences in lengths as the season progressed.

RESULTS

Mark-Recapture

Tag retention and mortality associated with the tagging procedure were examined in the laboratory prior to using the method in the field. Twenty-eight randomly selected staghorn sculpin were tagged and held in aquaria for one month. The capture and tagging methods were the same as described in the Methods section. One fish lost its tag (96.4% retention rate) and no sculpin died as a result of the tagging process.

Staghorn sculpin were the most abundant fish captured during beach seining events in all months and in both plot types (Figure 1.5). On average from May through August, 74% of the total catch on cultured plots and 79% of the total catch on reference plots were staghorn sculpin. From 2011-2012, a total of 2681 sculpin were tagged at all sites and plots (Table 1.4). Recapture rates were slightly higher on the reference plots and ranged from 1.0-3.6%, while rates on the cultured plots were 0.0-2.7%. The consistently low percentage of recaptures did not allow for calculation of disappearance rates of staghorn sculpin. Schnabel estimates (Schnabel 1938) for the reference plots suggested population sizes of 2568 (Foss), 5390 (Manke) and 10453 (Rolfs), however the estimates could be biased high due to the low recapture rate. There were 71 total recaptures, of which 70 were sculpin caught on the same plot as originally captured. The results were consistent with site fidelity, and it was concluded that staghorn sculpin exhibited fidelity to cultured or reference plots at the study sites and did not move between plots to forage.

Stomach Content Analysis

Table 1.2 and Figure 1.4 show the average mass-based proportions of all prey groups for each plot and month. In general, the most dominant prey groups found in the gut contents were AMPH and CRAB, with CORO, ISO, POLY and SHRI showing higher proportions during specific month-plot combinations (Figure 1.4). BIV, OCRUST and OTH presented low proportions overall. Polychaetes and amphipods were consumed earlier in the season (May-July), while crabs generally increased in proportion with each subsequent month and dominated the stomach contents during the months of July-September at both plots. CRAB and SHRI had higher proportions on the cultured plots, while the CORO and ISO groups were commonly associated with the reference plots.

ANOSIM detected significant differences in sculpin gut contents between plots in the months of June, July, August and September (Table 1.5). There was no statistically significant difference between plots in the month of May. To aid in visualization of the data, two dimensional NMDS plots were created for each of the five months (Figures 1.6-1.10). Vector overlays of significant prey groups ($p < 0.05$) showed associations of different food items with the two plot types. Representative of the strength of statistically significant differences between plots, NMDS displayed strong intermixing of sculpin gut contents between plots for May (Figure 1.6), moderate to low intermixing for June and August (Figures 1.7 and 1.9) and clear separation of plots in July and September (Figures 1.8 and 1.10). The reference plots were generally more associated with corophium amphipods, isopods and other crustaceans (Figures 1.7-1.10), while the cultured plots were more often linked to bivalves and crabs (Figures 1.7, 1.8 and 1.10).

Diet comparisons between each month pairing were conducted for cultured and reference plots, resulting in ten ANOSIM tests per plot (as detailed in Table 1.6). For the cultured plots, ANOSIM detected significant differences in seven of the ten month pairings, and for reference plots in six of the ten (Table 1.6). NMDS plots for May and September at both habitat types demonstrate the variability in sculpin diets between the beginning and the end of the study period (Figures 1.11 and 1.12). Overall, NMDS of cultured and reference plots show increased consumption of amphipods and isopods in the month of May, while crabs were more commonly eaten in September.

Stable Isotope Analysis

Mean stable carbon and nitrogen isotope signatures are summarized in Table 1.7 and presented as biplots by month (Figures 1.13-1.15) and by organism (Figure 1.16-1.22). While there was a high degree of variability within some of the prey group isotope signatures and broad similarity in signatures between plot types, some general patterns were apparent. Throughout months, the overall pattern of $\delta^{15}N$ values showed sculpin with the most enrichment, generally followed by PPOLY, FPOLY, SHRI and CRAB, then CORO and AMPH (Figures 1.13-1.15). Standard errors for staghorn sculpin signatures were low. Corresponding with growth, sculpin became more enriched in $\delta^{15}N$ as the season progressed (Figure 1.16).

In April, several prey groups displayed slight enrichment on the reference plots: AMPH, CORO and FPOLY were more enriched in $\delta^{13}C$, and FPOLY and PPOLY were more enriched in $\delta^{15}N$ compared to the cultured plots (Figure 1.13). Interestingly, the PPOLY group in April showed similar $\delta^{15}N$ level as staghorn sculpin in May. At the reference plots in June, the AMPH group was more enriched in $\delta^{15}N$ and the PPOLY group was slightly more enriched in $\delta^{13}C$

(Figure 1.14) compared to the cultured plots. Conversely, at the reference plots in August, the CRAB and SHRI groups were more depleted in $\delta^{13}C$ than at the cultured plots (Figure 1.15).

AIC values for linear mixed effects models and Akaike parameter weights for stable isotope data from staghorn sculpin are summarized in Tables 1.8-1.9 for $\delta^{13}C$ and Tables 1.10-1.11 for $\delta^{15}N$. Models 25 and 26 for each response variable did not converge and were excluded from the candidate set. Carbon and nitrogen analyses each resulted in ten top models, with cumulative Akaike weights of 0.95 or less, which were used to calculate parameter weights for length, month, plot and applicable interaction terms.

The strongest candidate model for carbon was the month only model, followed by five other models with ΔAICc values <2: plot x length, plot x month, length only, plot x length + month and plot + month (Table 1.8). There was a 21% chance that the month only model was the model of best fit, a 14% chance that model 2 was best, etcetera (Table 1.8). Parameter weights for carbon showed no effect of length, month, plot or the interaction terms (Table 1.9).

For nitrogen, the strongest candidate model was month + length, followed by the models for length only and month only (Table 1.10). The top three models had 59% probability of including the best model. Parameter weights suggested no effect of length, month, plot or the interaction terms (Table 1.11).

Stable Isotope Mixing Model

The SIAR mixing model was run for cultured and reference plot staghorn sculpin for the months of May, July and September, with associated source/prey group data from April, June and August, respectively. The median proportion (Figure 1.23) and interquartile range of each prey group, as well as the unknown source contribution are detailed in Table 1.12. The UNKN and AMPH contributions were low (<10%) for all months. In May, CORO proportions were high (>50%) for both plots and SHRI and POLY were other major contributors to the cultured and reference plots, respectively. In July, CRAB and POLY proportions were high for both plots and SHRI were also important in the diets of sculpin from the cultured plots. CORO and SHRI were important in September at the cultured and reference plots, respectively, while POLY demonstrated fairly high proportions at both plots.

The SIMM results somewhat contradicted the SCA findings. Mixing model proportions of AMPH were very low, while AMPH was a significant component of the gut contents. CRAB was another dominant group in the SCA, especially later in the season, but SIAR showed low proportions of CRAB except during the month of July. Conversely, the POLY group resulted in higher proportions overall in the mixing model compared to SCA. The only prey group that showed a somewhat similar pattern between SIAR and SCA was SHRI. However, at the reference plots in September, the pattern is drastically different in that SHRI were a low component of the gut contents and a very high proportion of the SIMM prediction.

Fitness Metrics Analyses

There were no significant differences between plots or months in the ratio of sculpin mass to the mass of the stomach contents (Figure 1.24, Table 1.13). The plot to month interaction term was also non-significant. Thus, gut fullness was similar between cultured and reference plots throughout the field season.

For the ratio of sculpin length to sculpin mass, there were no significant differences between plots within any month (Figure 1.25, Table 1.14). For this metric, a lower ratio corresponds to an increase in body condition. The lower ratio later in the season is not necessarily indicative of improved body condition since sculpin in September were larger than those in May.

DISCUSSION

Pacific staghorn sculpin are an integral component of nearshore ecosystems of Puget Sound where geoduck aquaculture takes place. Overall, the study showed no unequivocal effect of the structured phase of geoduck aquaculture on the trophic ecology of staghorn sculpin. Geoduck aquaculture produced changes that were reflected in some aspects of trophic dynamics, such as sculpin diets, but the trophic position of sculpin and the source of primary production within their food webs was unchanged. Thus, the overall function and chemical structure of the ecosystem remained intact despite disturbances associated with geoduck culture operations, within the conceptual limits defined by study design.

There were patterns related to differences between cultured and reference plots in the diets of staghorn sculpin. For example, crabs and shrimp were consumed in higher proportions at the cultured plots, while corophium amphipods and isopods were more commonly consumed at the reference plots. ANOSIM detected statistically significant differences in diet between plots in June through September. However, the associated R values were generally small, indicating a low to moderate level of ecological significance, depending on the month. The biggest difference in sculpin diet between plots occurred in the month of September, which was dominated by crab at the cultured plots and corophium amphipods, crabs and isopods at the reference plots.

Plot differences in the diets of staghorn sculpin were not unexpected given the results of previous research. Several studies have established that habitat complexity can initiate changes in the community composition of sculpin prey groups, resident infauna and epifauna (see Dumbauld et al. 2009). Spencer et al. (1997) found that organic enrichment from netting used in clam aquaculture changed dominant infaunal taxa. McDonald et al. (in press) noted some specific differences in resident infaunal and epifaunal populations at Puget Sound geoduck aquaculture sites in the structured phase. The amphipod *Americorphium salmonis* (part of the corophium amphipod sculpin prey group) was found in lower abundance at aquaculture sites compared to nearby reference areas. The same study also found differences in the types of macroinvertebrates utilizing cultured and reference areas. In particular, crabs were more strongly associated, numerically, with the aquaculture areas. The patterns for *Americorphium salmonis* and crabs in the McDonald et al. study corroborate some of the differences in staghorn sculpin diets seen here. Conversely, Brown and Thuesen (2011) caught larger numbers of graceful crab, *Metacarcinus gracilis* (formerly *Cancer gracilis*), in traps at a control area compared to a structured geoduck aquaculture site in Puget Sound. However, crabs less than 12.7 mm (a size more suitable for consumption by sculpin) were excluded from the Brown and Thuesen study and many crab species are known to exhibit ontogenetic shifts in habitat (see Holsman et al. 2006).

Differences in diets between distinct habitats were reflected in the gut contents of demersal fishes from other studies as well. López-Jamar et al. (1984) found significant differences in the diets of three types of demersal fish inhabiting areas dominated by mussel rafts compared to areas with minimal mussel aquaculture in Spain. For all species, gut contents were dominated by crabs in the concentrated aquaculture habitat, compared to a diet of benthic infauna in the low-intensity aquaculture areas. Gillanders (1995) found that a benthic species of grouper (Perciformes, Serranidae, Epinephelinae) near Sydney, Australia had different diets in sea grass habitat compared to nearby rock reef environments. In another study of staghorn sculpin consumption in Grays Harbor, WA, Armstrong et al. (1995) found significant differences in sculpin diet between bivalve shell and eelgrass habitats, with crab dominating diet in the shell habitat.

The mixing model SIAR also showed some differences between plots in sculpin diets, especially in September, however different prey groups emerged as important compared to SCA. Polychaetes were in higher proportion and crabs had lower representation in the mixing model than in the gut contents. Given differences in the digestion rates of prey, it is likely that polychaetes were at least somewhat underrepresented and crabs were over-represented to some extent in the gut contents. Polychaetes would be quickly processed and less easy to detect and identify in gut contents. On the other hand, crabs have more hard parts that are not immediately evacuated from the gut (see Hyslop 1980), which may reduce the food value of crabs per unit of body mass. Another potential explanation for the discrepancy between SCA and SIAR is that staghorn sculpin may utilize subtidal areas for foraging when tides are low. While the stomachs of sculpin captured during flood tides were previously found to be mostly empty (R. Smith and P.S. McDonald, unpublished data), it is still plausible that sculpin occasionally forage subtidally, which would account for some difference in the results of SCA and SIAR. Nonetheless, as with other methods for estimating diet, there are limitations to using stable isotope mixing models (Parnell et al. 2010) and the prey groups here may not have distinct enough stable isotope signatures to accurately represent all aspects of sculpin diet in a SIMM (e.g. Polito et al. 2011).

Between months within the same plot type, there were low to moderate differences in sculpin gut contents detected by ANOSIM. Differences were larger between early and later months, corresponding to seasonal changes in the prey base and a change in diet due to increasing sculpin body size and mouth gape. In May, more isopods were consumed, while more crabs were eaten in September. Tasto (1975) also noted a change in the diet of staghorn sculpin related to growth and season, with a shift from consumption of small invertebrates to more crabs.

Seasonal changes are also common in the sculpin prey base. McDonald et al. (in press) found significant temporal differences in the abundance of resident infaunal and epifaunal taxa at structured geoduck aquaculture sites in Puget Sound. Clear seasonal patterns were also noted in infaunal abundance in a Connecticut estuary (Zajac and Whitlatch 1982) and in a Massachusetts marsh (Sardá et al. 1995). General observations of algal growth on geoduck aquaculture structures in parts of the summer could also alter infauna and epifauna at the cultured plots (e.g. Everett 1991). While the McDonald et al. study did not detect a significant effect of geoduck aquaculture on the structure of the infaunal community as a whole, there were shifts in individual taxa, including some that appeared in the diets of staghorn sculpin.

Compared to the snapshot diet information provided by SCA, stable isotopes better represent fundamental changes in energy flow through an ecosystem. Differences between cultured and reference plots in sculpin diet were not clearly seen on a chemical level in sculpin stable isotope signatures. For stable carbon and nitrogen, there were ten top statistical models, from an original list of 26. The large group of top models with cumulative Akaike weights <0.95 suggest no overwhelming effect of any single factor. Any one of the top ten models could provide reasonable explanatory power to the dataset (Symonds and Moussalli 2011). Plot was included in over half of the top models for carbon and nitrogen, suggesting that plot explained some of the variability in the stable isotope data. However, the effect of plot was low since the model-averaged weight of plot showed no effect for carbon or nitrogen. The parameter weights also showed no effect of length, month or applicable interaction terms.

Based on the model-averaged parameter weight, there was a near effect of length on nitrogen, suggesting that sculpin length may explain more of the variability for nitrogen than plot or month. Length and month were included in the top three models for nitrogen. The two factors are tightly correlated since sculpin were larger later in the season. Ecologically, an effect of fish length on nitrogen is expected since organisms become more enriched in nitrogen as they consume more enriched prey and increase in trophic level (Minagawa and Wada 1984). For example, increased age, weight and length were shown to enrich stable nitrogen signatures of walleye in a Vermont lake (Overman and Parrish 2001). If sculpin sampling took place over a longer time interval than four months, the length range would be broader and a length effect on nitrogen might be more evident. The lack of differences between cultured and reference plots in the stable isotope data were corroborated by the results of both sculpin fitness metrics, which showed no differences between the two habitat types in sculpin gut fullness or body condition. Metrics such as gut fullness have been used in many ecological studies researching differences in fish health between habitats. For instance, data from SCA determined that diet and gut fullness of several fish species were similar in mudflat and mangrove habitat, suggesting that both habitat types were important for conservation efforts in China (Tse et al. 2008).

Stomach contents are frequently used to provide resolution to stable isotope data and differences in diets are not always reflected as structural changes within a food web. For example, SCA showed that a non-native species of catfish in South Africa had distinct diets in three disparate habitats, however stable carbon and nitrogen signatures were similar among sites (Kadye and Booth 2011). In a study of fishes of the Chukchi Sea, SCA revealed that Arctic staghorn sculpin (*Gymnocanthus tricuspis* Reinhardt, 1830) had different diets based on location and stable nitrogen showed increased enrichment with sculpin length, but no differences in stable isotope signatures were seen between sites (ConocoPhillips Alaska, Inc. 2011). Stable isotopes also indicated that the feeding patterns of fishes in a Mediterranean marine food web were more diverse than indicated by SCA alone (Pinnegar and Polunin 2000). Such results emphasize both the practicality of SIA and the limitations of SCA, which reveals diet at only a very specific time and cannot accurately represent diets that are variable over short time spans. A change in $\delta^{15}N$ between trophic levels is equivalent to at least three per mil and up to five per mil (Peterson and Fry 1987). When accounting for variation, even a three per mil difference was not seen between plots in staghorn sculpin or sculpin prey groups. Differences in diet between habitats will not relate to a shift in a consumer's trophic position if dominant prey taxa are within the same level of a food web.

Primary producers have distinct isotope signatures that are reflected in the $\delta^{13}C$ values of each member of a food web. For example, food webs based phytoplankton versus macroalgae would exhibit $\delta^{13}C$ differences of a minimum of three per mil and upwards of ten per mil (Coleman and Fry 1991, France 1995). In a Brazilian estuary, large differences in carbon signatures (>10‰) were found between primary producers of mudflat and marsh creek habitats, with the mudflat habitat significantly more enriched in carbon (Claudino et al. 2013). Based on staghorn sculpin and their primary prey groups, there was no evidence in the stable isotope data to suggest that geoduck aquaculture initiated a shift in the source of primary production, a somewhat surprising finding. There was a visually striking presence of macroalgae on the aquaculture structures during the summer months of the study (personal observation, Figure 1.1) that was generally lacking in adjacent, sandy-bottom areas. At existing levels, algal growth apparently did not support development of a new food web.

Changes to primary production from biodeposition comprise another aspect of bivalve aquaculture that often raises concern due to the high density of cultured organisms (see Newell 2004 and Dumbauld et al. 2009). Wild populations of geoducks exist subtidally from 0-22.5 clams/m^2, with an average density of 1.7 clams/m^2 (Goodwin and Pease 1991). In intertidal farmed areas, densities at outplanting are 20-30 geoducks/m^2 (VanBlaricom et al. in press). Bivalve feces and pseudofeces settle and accumulate on the sediment surface, increasing or decreasing nitrogen levels (and thus promoting or reducing phytoplankton production), depending on the specific aquatic chemistry of a system (see Newell 2004). High densities of filter-feeding bivalves may lower water turbidity and increase growth of aquatic plants, such as benthic algae, that may dominate primary production (Newell and Koch 2004). In areas with low mixing, extremely high rates of biodeposition can reduce sediment oxygen content, resulting in anoxic conditions and an accumulation of toxic hydrogen sulfide (Diaz and Rosenberg 1995). However, in areas with frequent wave activity, such as the intertidal, nitrogen biodeposits are more likely to become resuspended in the water column, which increases dissolved nitrogen content and promotes production of both phytoplankton and benthic microalgae (Kaspar et al. 1985, Asmus and Asmus 1991, Dame et al. 1991). Biodeposition from cultured geoducks was not profound enough to alter primary production of the food web in which sculpin operate, an outcome that could be dependent on mixing from frequent wave disturbances at the study sites (see VanBlaricom et al. in press).

While SIA did not detect ecologically significant differences between habitats here, the method has proven robust for studying similar systems and many aquatic studies use SIA to investigate food web dynamics in complex habitats. Abeels et al. (2012) used SIA to elucidate trophic relationships at oyster reefs in Florida: resident and transient fishes had distinct isotopic signatures that gave insight into the importance of oyster reef habitat for refuge and foraging. Similarly, Yeager and Layman (2011) determined that a highly abundant species of goby (Perciformes, Gobiidae) in a Florida estuary was a significant link in the transfer of energy within oyster reef habitat and a critical prey resource for a commercially important species of snapper. Carbon isotopes in a study in Kenya showed that nearshore fishes exhibited foraging preference for seagrass beds over adjacent mangrove habitat (Marguillier et al. 1997). Snapper (Perciformes, Lutjanidae) in Japan favored mangrove forests as nursery habitat and utilized coral reefs as adults, a finding corroborated both by SCA and SIA (Nakamura et al. 2008). The food web of staghorn sculpin at geoduck aquaculture sites was not distinct enough, based on the data reported here, to alter the carbon or nitrogen signatures of sculpin or primary sculpin prey groups.

Results from this study will inform decisions to better balance economic needs with those of maintaining natural ecosystems and aid management agencies in making regulatory decisions relevant to the geoduck aquaculture industry. The current scale of geoduck culture initiated some changes to trophic dynamics, however the impacts detected were not significant enough to alter energy flow or the overall structure of the food web of staghorn sculpin. The community structure of demersal fishes in Puget Sound is known to change seasonally, with summer being the season of greatest prey availability and diet overlap (Reum and Essington 2008). Trophic impacts from the structured phase of geoduck aquaculture could be further minimized by outplanting larger geoducks, reducing the amount of time nets and tubes are in place during ecologically productive summer months and removing gear as early in the farming cycle as possible. In addition, baseline monitoring of resident and transient fauna should be prioritized if operations were to expand.

It is important to note that the present study is based on data from three non-adjacent geoduck aquaculture sites during the course of one farming cycle. The results cannot be extrapolated to forecast the impacts of geoduck aquaculture operations in close proximity or repeated farming activities in the same location (see McDonald et al. in press, VanBlaricom et al. in press, Dumbauld et al. 2009). With increasing density, geoduck aquaculture may significantly impact trophic dynamics in areas of Puget Sound to the point of considerably changing the results presented here. For example, primary production could shift over the course of many culture cycles and more drastically change the composition of resident infauna and epifauna upon which sculpin feed, subsequently altering the trophic position of nearshore fishes and the dynamics of the entire food web. In addition, while staghorn sculpin populations do not currently display obvious deleterious impacts from geoduck aquaculture and some organisms will benefit from the habitat complexity of aquaculture gear, the presence of structures will reduce preferred habitat and foraging efficiency of certain organisms, such as flatfishes, that are adapted to soft-bottom environments (e.g. Woodin 1981, Holsman et al. 2010, McDonald et al. in press). It is imperative that research keep pace with the expansion of aquaculture operations and continued focus is placed on balancing human needs with preserving nearshore marine ecosystems.

Table 1.1. Field site descriptions. MLLW: mean lower low water.

Site	Location	Tidal Range	Substrate (Grain size)	Common Biota
Foss	Case Inlet, west of Joemma Beach State Park	+0.61 m to -0.61 m MLLW	Sandy (~500 µm)	horse clam (*Tresus* spp.), cockle (*Clinocardium nutalli*), graceful crab (*Metacarcinus gracilis*), northern kelp crab (*Pugettia producta*), and Pacific sand dollar (*Dendraster excentricus*)
Manke	Case Inlet, south of Wilson Point on Harstine Island	+0.61 m to -0.61 m MLLW	Sandy with some fines (~250-500 µm)	
Rolfs	Case Inlet, north of Wilson Point on Harstine Island	+0.76 m to -0.61 m MLLW	Sandy with some fines (~250-500 µm)	

Table 1.2. Diet composition as the proportional weight contribution of each prey group from 2012 sculpin stomach contents by month and plot. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, OTH: other, POLY: polychaetes, SHRI: shrimp.

Month	Plot	AMPH	BIV	CORO	CRAB	ISO	OCRUST	OTH	POLY	SHRI
May	CULT	0.568	0.005	0.093	0.000	0.171	0.002	0.025	0.043	0.093
May	REF	0.262	0.000	0.179	0.042	0.133	0.012	0.082	0.291	0.000
June	CULT	0.345	0.002	0.041	0.371	0.065	0.004	0.001	0.115	0.056
June	REF	0.255	0.000	0.058	0.229	0.368	0.010	0.022	0.057	0.001
July	CULT	0.038	0.002	0.005	0.625	0.032	0.015	0.008	0.105	0.170
July	REF	0.062	0.000	0.249	0.500	0.105	0.047	0.003	0.032	0.002
Aug	CULT	0.034	0.000	0.004	0.839	0.041	0.000	0.058	0.000	0.024
Aug	REF	0.151	0.000	0.070	0.707	0.010	0.005	0.001	0.000	0.055
Sep	CULT	0.010	0.000	0.011	0.915	0.003	0.001	0.014	0.001	0.045
Sep	REF	0.093	0.001	0.124	0.519	0.108	0.019	0.055	0.019	0.062

Table 1.3. Diet proportions used in the mixing model, SIAR. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, POLY: polychaetes, SHRI: shrimp.

Month	Plot	AMPH	CORO	CRAB	POLY	SHRI
May	CULT	0.713	0.116	0.000	0.054	0.116
May	REF	0.339	0.231	0.055	0.376	0.000
July	CULT	0.040	0.006	0.663	0.112	0.180
July	REF	0.073	0.295	0.592	0.038	0.002
September	CULT	0.010	0.011	0.932	0.001	0.046
September	REF	0.113	0.152	0.635	0.024	0.076

Table 1.4. Numbers of captured/tagged (≥65 mm) and recaptured staghorn sculpin by year, site and plot. * Indicates location of one fish that was not recaptured on the same plot as original capture.

Site	Plot	Year	# Sculpin Captured/Tagged	# Recaptures	Recapture %
Foss	CULT	2011	296	8	2.7
Foss	REF	2011	290	10	3.4
Foss	CULT	2012	132	2*	0.7
Foss	REF	2012	459	16	3.5
Manke	CULT	2012	395	10	2.5
Manke	REF	2012	581	21	3.6
Rolfs	CULT	2012	116	0	0.0
Rolfs	REF	2012	412	4	1.0
Total			2681	71	

Table 1.5. ANOSIM summary statistics for tests conducted on stomach content data between cultured and reference plots for each month. Bold indicates statistically significant difference with $p<0.05$.

Test Between Plots	ANOSIM R	p-value
May	-0.005	0.504
June	0.042	**0.034**
July	0.177	**0.001**
Aug	0.055	**0.034**
Sep	0.283	**0.001**

Table 1.6. ANOSIM summary statistics for tests conducted on stomach content data between each month pairing for cultured and reference plots. Bold indicates statistically significant difference with $p<0.05$.

Test Months	Plot	ANOSIM R	p-value
May and June	CULT	0.033	0.111
May and June	REF	0.014	0.215
May and July	CULT	0.083	**0.011**
May and July	REF	0.110	**0.001**
May and Aug	CULT	0.186	**0.001**
May and Aug	REF	0.145	**0.001**
May and Sep	CULT	0.443	**0.001**
May and Sep	REF	0.105	**0.001**
June and July	CULT	0.000	0.438
June and July	REF	0.043	0.055
June and Aug	CULT	0.069	**0.014**
June and Aug	REF	0.047	**0.046**
June and Sep	CULT	0.245	**0.001**
June and Sep	REF	0.056	**0.026**
July and Aug	CULT	0.028	0.123
July and Aug	REF	0.083	**0.003**
July and Sep	CULT	0.132	**0.001**
July and Sep	REF	0.027	0.093
August and Sep	CULT	0.048	**0.045**
August and Sep	REF	0.024	0.129

Table 1.7. Mean $\delta^{13}C$ and $\delta^{15}N$ values and standard error (SE) for all organisms. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, FPOLY: filter-feeding polychaetes, PPOLY: predatory polychaetes, SHRI: shrimp, SCULP: staghorn sculpin.

Group	Month	Plot	N	Mean $\delta^{13}C$	SE $\delta^{13}C$	Mean $\delta^{15}N$	SE $\delta^{15}N$
AMPH	April	CULT	5	-13.798	0.450	7.905	0.302
AMPH	April	REF	5	-10.372	0.666	8.037	0.327
AMPH	June	CULT	5	-12.560	0.343	7.905	0.085
AMPH	June	REF	2	-13.266	0.568	9.535	0.102
AMPH	Aug	CULT	5	-11.622	0.483	9.206	0.215
AMPH	Aug	REF	5	-11.917	0.147	9.357	0.312
CORO	April	CULT	5	-15.706	0.713	9.300	0.196
CORO	April	REF	5	-13.152	0.219	8.730	0.216
CORO	June	CULT	4	-15.559	0.421	9.085	0.159
CORO	June	REF	5	-14.880	0.311	8.874	0.159
CORO	Aug	CULT	2	-16.199	0.755	9.454	0.308
CORO	Aug	REF	5	-15.182	0.418	9.330	0.165
CRAB	April	CULT	5	-10.047	1.050	8.593	0.884
CRAB	April	REF	2	-8.679	1.909	9.091	0.142
CRAB	June	CULT	5	-14.158	0.481	10.049	0.085
CRAB	June	REF	2	-15.115	0.159	9.678	0.197
CRAB	Aug	CULT	5	-10.533	0.224	10.594	0.180
CRAB	Aug	REF	5	-12.950	0.258	10.487	0.186
FPOLY	April	CULT	5	-13.610	0.321	11.095	0.442
FPOLY	April	REF	5	-11.457	0.387	12.628	0.512
FPOLY	June	CULT	5	-13.446	0.554	11.200	0.910
FPOLY	June	REF	5	-14.093	1.223	10.950	0.849
FPOLY	Aug	CULT	5	-13.318	0.846	12.486	0.506
FPOLY	Aug	REF	5	-12.363	0.728	12.311	0.738
PPOLY	April	CULT	5	-12.978	0.540	13.078	0.419
PPOLY	April	REF	5	-12.418	0.474	14.812	0.707
PPOLY	June	CULT	5	-13.057	0.301	12.637	0.567
PPOLY	June	REF	5	-11.606	0.643	12.919	0.362
PPOLY	Aug	CULT	5	-11.258	0.607	13.129	0.364
PPOLY	Aug	REF	5	-11.673	0.779	13.563	0.631
SHRI	April	CULT	5	-12.378	0.184	10.923	0.360
SHRI	June	CULT	5	-12.715	0.381	11.598	0.199
SHRI	Aug	CULT	5	-12.610	0.437	11.183	0.137
SHRI	Aug	REF	2	-15.479	0.681	10.765	0.021
SCULP	May	CULT	15	-13.608	0.277	13.572	0.103
SCULP	May	REF	15	-12.989	0.240	13.801	0.166
SCULP	July	CULT	15	-14.174	0.270	14.501	0.132
SCULP	July	REF	15	-13.884	0.247	14.260	0.157
SCULP	Sep	CULT	15	-13.810	0.222	14.776	0.119
SCULP	Sep	REF	15	-14.346	0.242	14.702	0.125

Table 1.8. List of all candidate models for δ^{13}C with site included as a random effect on the intercept. L: staghorn sculpin length, M: month, P: plot, K: number of model parameters, ΔAICc: change in AICc, W_i: Akaike weight, ΣW_i: cumulative Akaike weight, ER: evidence ratio. Shaded models: included in list of top candidate models ($\Sigma W_i \leq 0.95$). Models 25 and 26 did not converge and were excluded from the analysis.

Model	Model ID	K	AICc	ΔAICc	Wi	ΣWi	ER
M	1	5	260.46	0	0.21	0.21	1.0
P + L + P x L	2	6	261.2	0.74	0.14	0.35	1.5
P + M + P x M	3	8	261.3	0.84	0.13	0.48	1.6
L	4	4	261.59	1.13	0.12	0.6	1.8
P + M + L + P x L	5	8	261.85	1.39	0.1	0.7	2.1
P + M	6	6	262.39	1.93	0.08	0.78	2.6
M + L	7	6	262.68	2.22	0.07	0.85	3.0
P + M + L + P x M	8	9	263.61	3.15	0.04	0.89	5.3
P + L	9	5	263.83	3.37	0.04	0.93	5.3
P + M + L	10	7	264.74	4.28	0.02	0.95	10.5
P + M + L + P x M + P x L	11	10	266.13	5.67	0.01	0.96	21.0
P + M + L + P x L + M x L	12	10	266.69	6.23	0.01	0.97	21.0
M + L + M x L	13	8	267.05	6.59	0.01	0.98	21.0
Null	14	3	267.41	6.96	0.01	0.99	21.0
P + M + L + P x M + M x L	15	11	268.39	7.93	0	0.99	N/A
P + M + L + M x L	16	9	269.25	8.79	0	0.99	N/A
P	17	4	269.28	8.82	0	1	N/A
P + M + L + P x M + P x L + M x L	18	12	271.06	10.6	0	1	N/A
P + M + L + M x L + P x M x L	19	12	271.47	11.01	0	1	N/A
P + M + L + P x L + P x M x L	20	12	271.47	11.01	0	1	N/A
P + M + L + P x L + M x L + P x M x L	21	12	271.47	11.01	0	1	N/A
P + M + L + P x M + M x L + P x M x L	22	14	272.92	12.46	0	1	N/A
P + M + L + P x M + P x L + M x L + P x M x L	23	14	272.92	12.46	0	1	N/A
P + M + L + P x M + P x L + P x M x L	24	14	272.92	12.46	0	1	N/A
P + M + L + P x M x L	25	N/A	N/A	N/A	N/A	N/A	N/A
P + M + L + P x M + P x M x L	26	N/A	N/A	N/A	N/A	N/A	N/A

Table 1.9. Model averaged parameter weights for $\delta^{13}C$.

Parameter	Base Model	Estimate	SE	Lower 95% CI	Upper 95% CI
Length	N/A	-0.006	0.006	-0.018	0.005
Month July	Month May	-0.411	0.265	-0.930	0.107
Month Sep	Month May	0.180	0.151	-0.116	0.476
Plot CULT	Plot Reference	-0.408	0.441	-1.273	0.458
Plot CULT x Length	Plot REF x Length	0.004	0.004	-0.003	0.011
Plot CULT x Month July	Plot REF x Month May	0.154	0.141	-0.122	0.430
Plot CULT x Month Sep	Plot REF x Month May	0.034	0.072	-0.107	0.175

Table 1.10. List of all candidate models for $\delta^{15}N$ with site included as a random effect on the intercept. L: staghorn sculpin length, M: month, P: plot, K: number of model parameters, ΔAICc: change in AICc, W_i: Akaike weight, ΣW_i: cumulative Akaike weight, ER: evidence ratio. Shaded models: included in list of top candidate models ($\Sigma W_i \leq 0.95$). Models 25 and 26 did not converge and were excluded from the analysis.

Model	Model ID	K	AICc	ΔAICc	Wi	ΣWi	ER
M + L	1	6	134.73	0	0.29	0.29	1.0
L	2	4	135.68	0.95	0.18	0.46	1.6
M	3	5	136.43	1.7	0.12	0.59	2.4
P + M + L	4	7	136.95	2.21	0.09	0.68	3.2
P + L	5	5	137.26	2.53	0.08	0.76	3.6
P + M + L + P x L	6	8	138.02	3.29	0.06	0.82	4.8
P + M	7	6	138.71	3.98	0.04	0.86	7.3
M + L + M x L	8	8	138.91	4.17	0.04	0.89	7.3
P + L + P x L	9	6	139.02	4.29	0.03	0.93	9.7
P + M + P x M	10	8	139.41	4.68	0.03	0.95	9.7
P + M + L + P x M	11	9	140.57	5.83	0.02	0.97	14.5
P + M + L + M x L	12	9	141.33	6.59	0.01	0.98	29.0
P + M + L + P x M + P x L	13	10	141.62	6.89	0.01	0.99	29.0
P + M + L + P x L + M x L	14	10	142.61	7.88	0.01	0.99	29.0
P + M + L + P x M + M x L	15	11	145.35	10.62	0	1	N/A
P + M + L + P x M + P x L + M x L	16	12	146.22	11.49	0	1	N/A
P + M + L + P x L + M x L + P x M x L	17	12	146.38	11.65	0	1	N/A
P + M + L + M x L + P x M x L	18	12	146.38	11.65	0	1	N/A
P + M + L + P x L + P x M x L	19	12	146.38	11.65	0	1	N/A
P + M + L + P x M + P x L + P x M x L	20	14	150.44	15.7	0	1	N/A
P + M + L + P x M + M x L + P x M x L	21	14	150.44	15.7	0	1	N/A
P + M + L + P x M + P x L + M x L + P x M x L	22	14	150.44	15.7	0	1	N/A
Null	23	3	188.51	53.78	0	1	N/A
P	24	4	190.68	55.95	0	1	N/A
P + M + L + P x M x L	25	N/A	N/A	N/A	N/A	N/A	N/A
P + M + L + P x M + P x M x L	26	N/A	N/A	N/A	N/A	N/A	N/A

Table 1.11. Model averaged parameter weights for $\delta^{15}N$.

Parameter	Base Model	Estimate	SE	Lower 95% CI	Upper 95% CI
Length	N/A	0.008	0.004	0.000	0.016
Month July	Month May	0.371	0.217	-0.055	0.797
Month Sep	Month May	-0.096	0.089	-0.270	0.079
Plot CULT	Plot REF	-0.059	0.105	-0.264	0.146
Plot CULT x Length	Plot REF x Length	0.000	0.000	-0.001	0.001
Month July x Length	Month May x Length	-0.001	0.001	-0.002	0.001
Month Sep x Length	Month May x Length	0.000	0.000	-0.001	0.001
Plot CULT x Month July	Plot REF x Month May	0.006	0.008	-0.009	0.021
Plot CULT x Month Sep	Plot REF x Month May	-0.007	0.009	-0.025	0.010

Table 1.12. Mixing model results: median proportions and interquartile range (IQR) of each prey group. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, POLY: polychaetes, SHRI: shrimp, UNKN: unknown.

Month	Plot		AMPH	CORO	CRAB	POLY	SHRI	UNKN
May	CULT	Median	0.011	0.537	0.000	0.037	0.381	0.034
		IQR	0.003-0.037	0.497-0.578	0.000-0.001	0.002-0.151	0.269-0.444	
May	REF	Median	0.007	0.653	0.000	0.304	0.000	0.036
		IQR	0.002-0.021	0.598-0.688	0.000-0.003	0.265-0.333	0.000-0.024	
July	CULT	Median	0.006	0.000	0.414	0.305	0.191	0.085
		IQR	0.001-0.023	0.000-0.077	0.271-0.517	0.198-0.396	0.026-0.405	
July	REF	Median	0.009	0.067	0.466	0.367	0.028	0.062
		IQR	0.001-0.032	0.021-0.150	0.344-0.557	0.298-0.416	0.001-0.131	
Sep	CULT	Median	0.000	0.464	0.009	0.484	0.007	0.035
		IQR	0.000-0.004	0.430-0.495	0.002-0.026	0.452-0.514	0.000-0.046	
Sep	REF	Median	0.000	0.009	0.001	0.183	0.790	0.016
		IQR	0.000-0.003	0.002-0.027	0.000-0.010	0.145-0.217	0.751-0.830	

Table 1.13. ANOVA table for the ratio of sculpin mass to the mass of the gut contents.

	Df	Sum Square	Mean Square	F-value	P-value
Plot	1	1.490	1.491	1.962	0.162
Month	4	2.210	0.553	0.727	0.574
Plot x Month	4	0.960	0.241	0.317	0.867
Residuals	308	234.080	0.760		

Table 1.14. ANOVA table for the ratio of sculpin length to sculpin mass. Months were tested independently to avoid bias due to allometric scaling.

	Df	Sum Square	Mean Square	F-value	P-value
<u>May:</u>					
Plot	1	0.003	0.003	0.071	0.791
Residuals	51	2.402	0.047		
<u>June:</u>					
Plot	1	0.0285	0.02845	0.479	0.493
Residuals	42	2.4949	0.0594		
<u>July:</u>					
Plot	1	0.0487	0.04871	1.186	0.286
Residuals	26	1.0684	0.04109		
<u>August:</u>					
Plot	1	0.071	0.07105	1.162	0.293
Residuals	22	1.345	0.06115		
<u>September:</u>					
Plot	1	0.0758	0.07579	1.989	0.166
Residuals	42	1.6001	0.0381		

Figure 1.1. Nets and tubes at a geoduck aquaculture site in South Puget Sound, Washington.

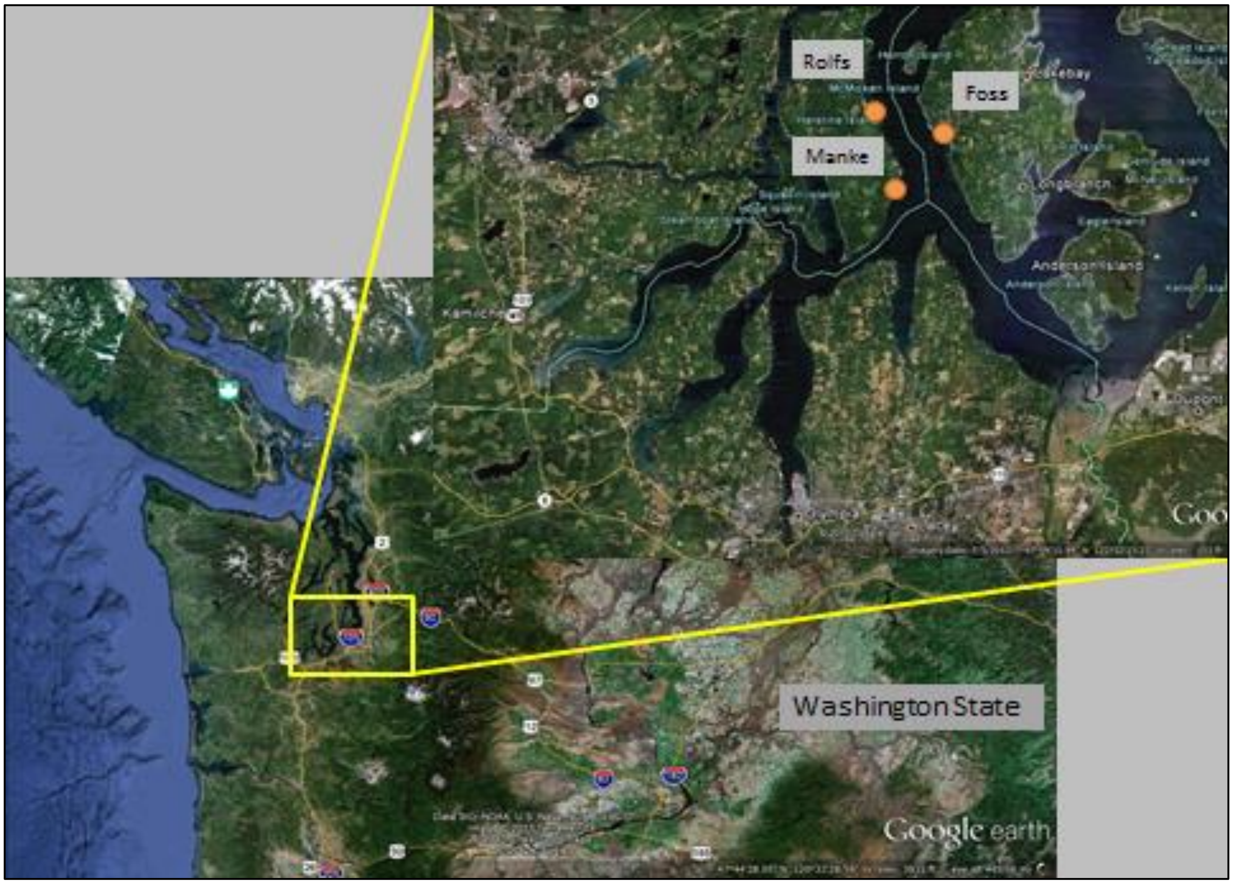

Figure 1.2. Location of research sites: Foss, Manke and Rolfs.

Pump handle

Outflow point with
removable sampling bag

Pump base with 150 micron mesh
covering the water intake area

Sampled area contained within the
opening under the pump base

Figure 1.3. Pumping device used to sample epibenthos.

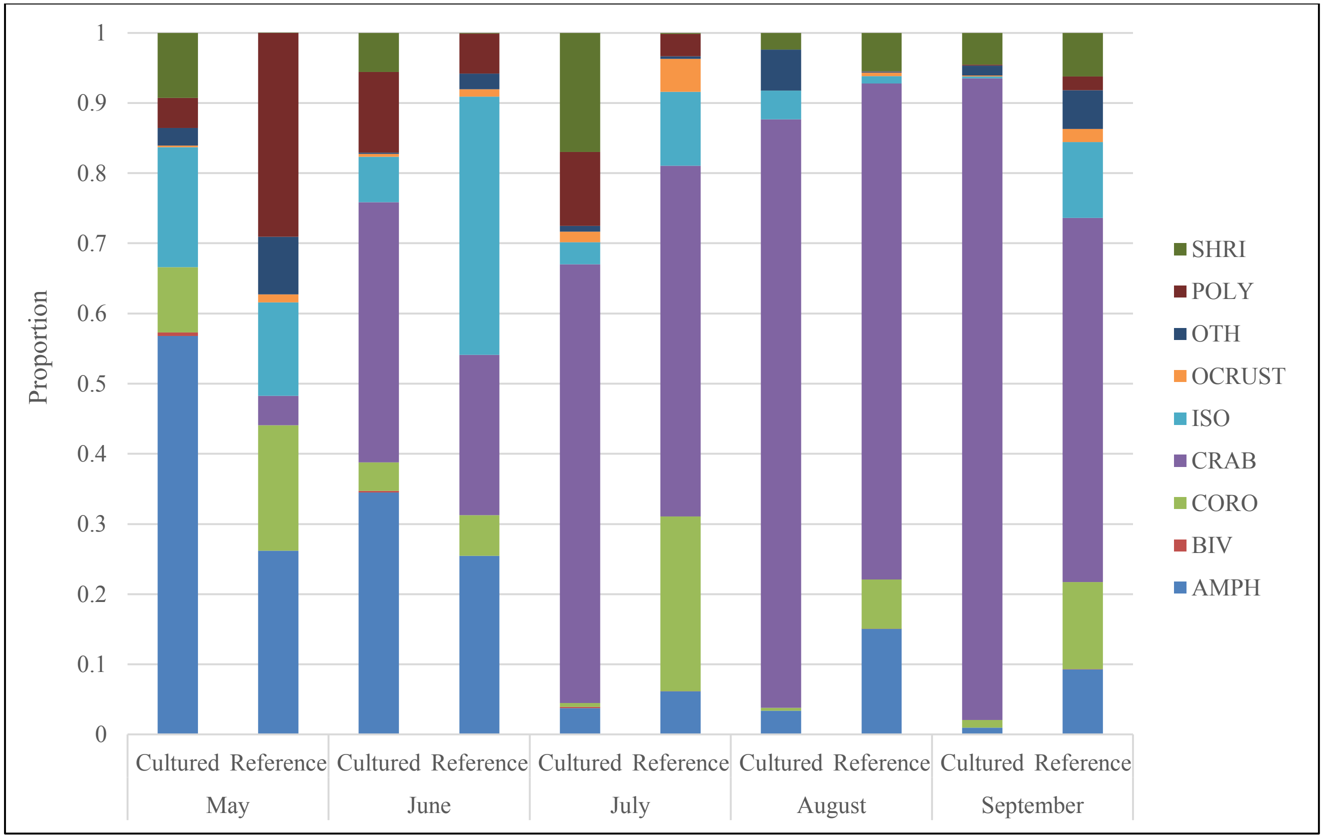

Figure 1.4. Average mass-based proportion of each prey group by month and plot from staghorn sculpin stomach contents. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, OTH: other, POLY: polychaetes, SHRI: shrimp.

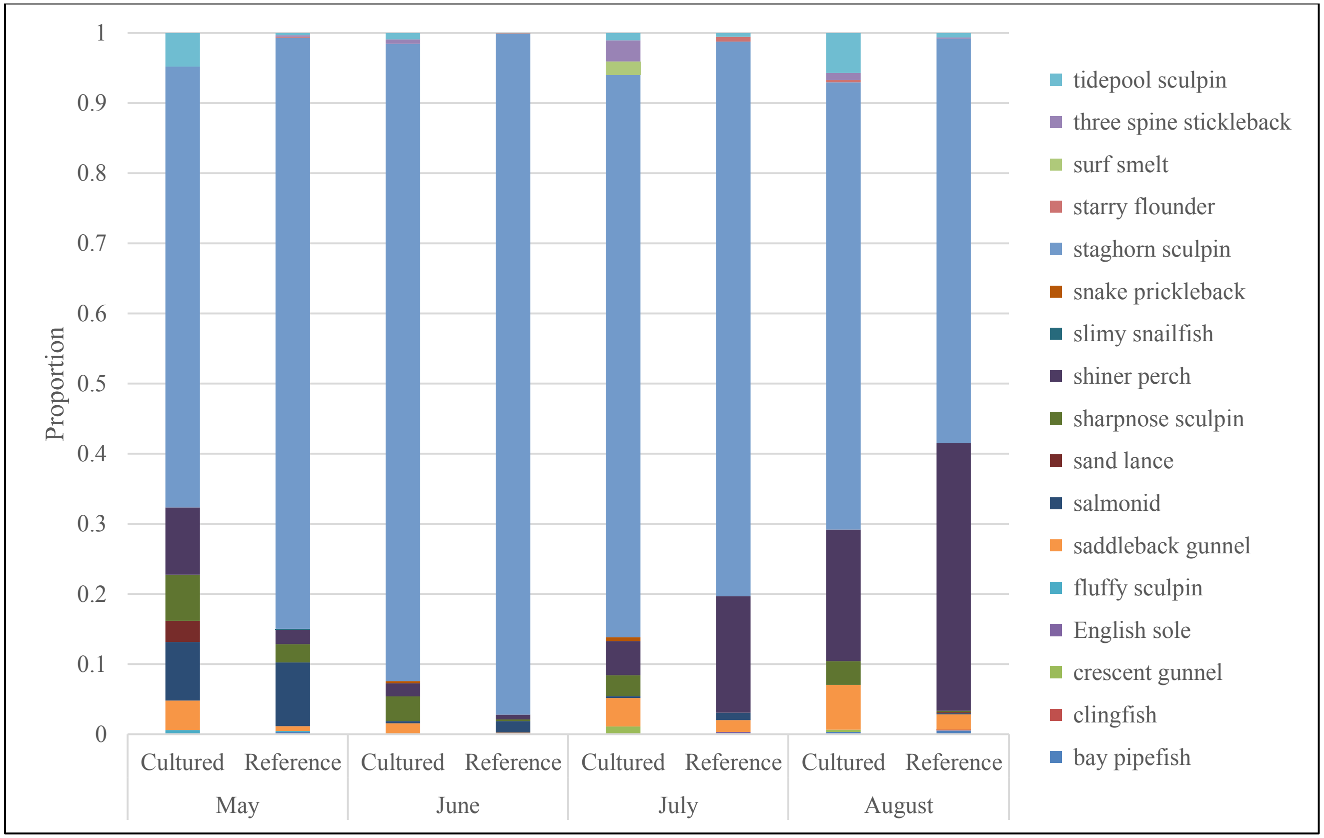

Figure 1.5. Relative abundances of all fish captured in beach seines at cultured and reference plots in May-August, 2012.

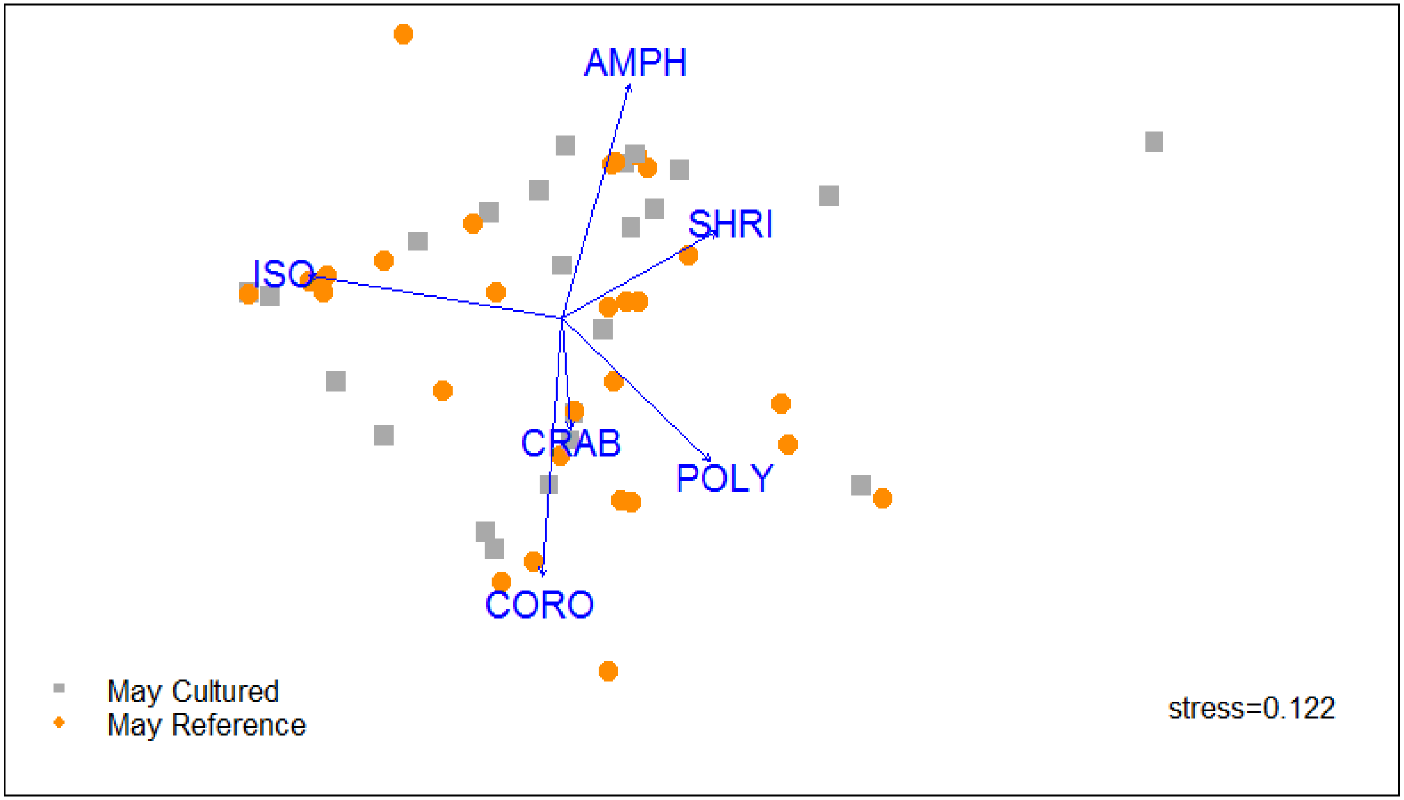

Figure 1.6. NMDS plot of staghorn sculpin stomach contents at cultured and reference plots in May. Significant prey groups ($p<0.05$) overlaid as blue vectors. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, POLY: polychaetes, SHRI: shrimp.

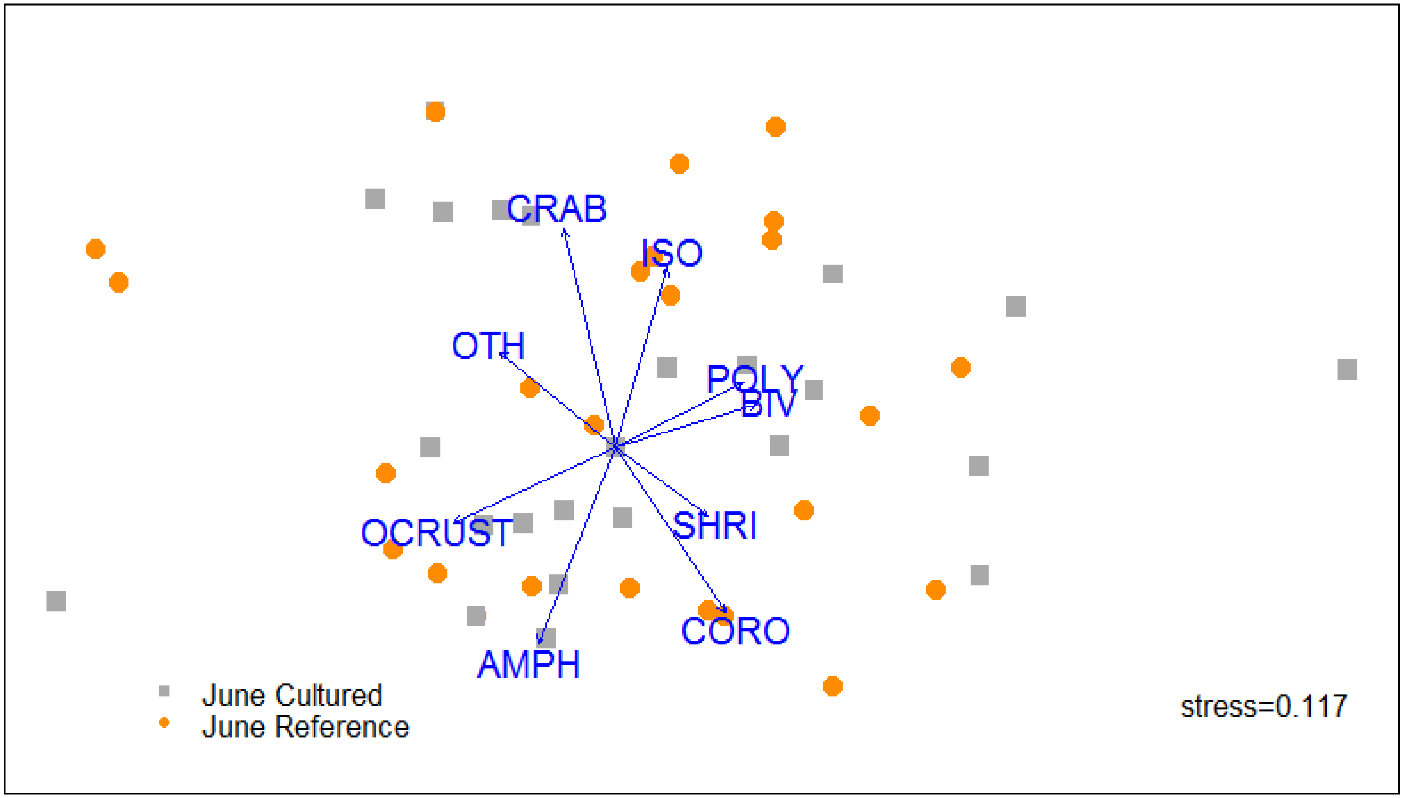

Figure 1.7. NMDS plot of staghorn sculpin stomach contents at cultured and reference plots in June. Significant prey groups ($p<0.05$) overlaid as blue vectors. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp.

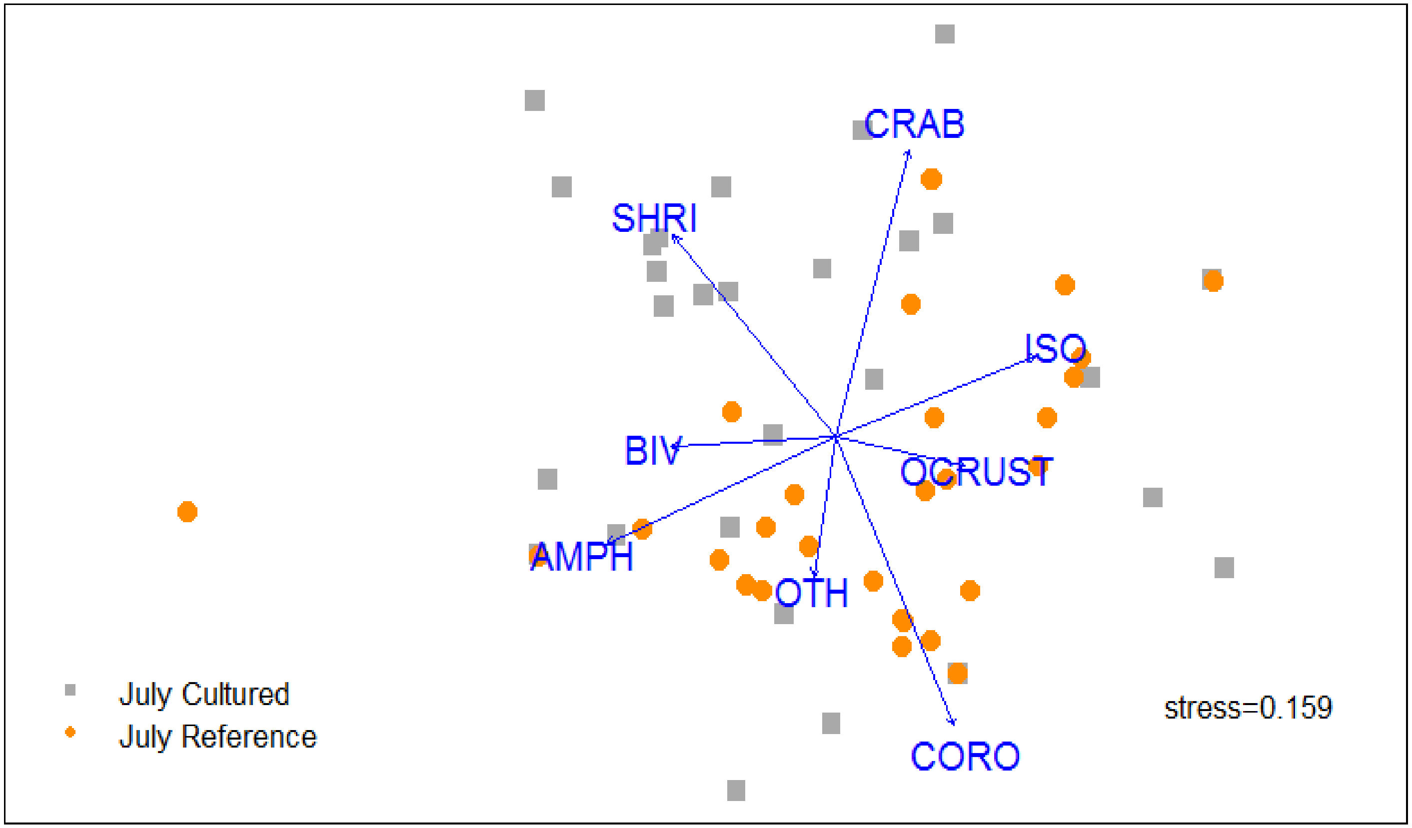

Figure 1.8. NMDS plot of staghorn sculpin stomach contents at cultured and reference plots in July. Significant prey groups ($p<0.05$) overlaid as blue vectors. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, SHRI: shrimp.

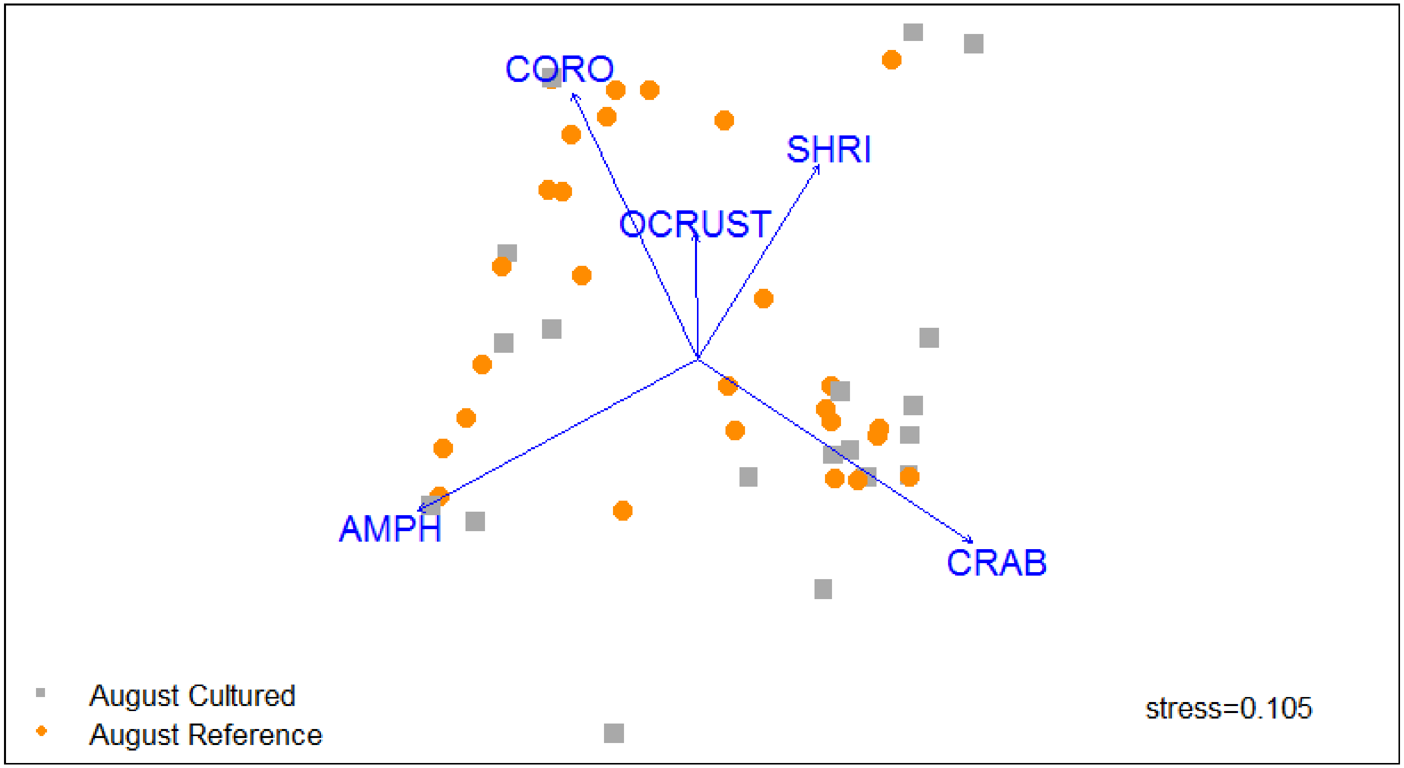

Figure 1.9. NMDS plot of staghorn sculpin stomach contents at cultured and reference plots in August. Significant prey groups ($p<0.05$) overlaid as blue vectors. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, OCRUST: other crustaceans, SHRI: shrimp.

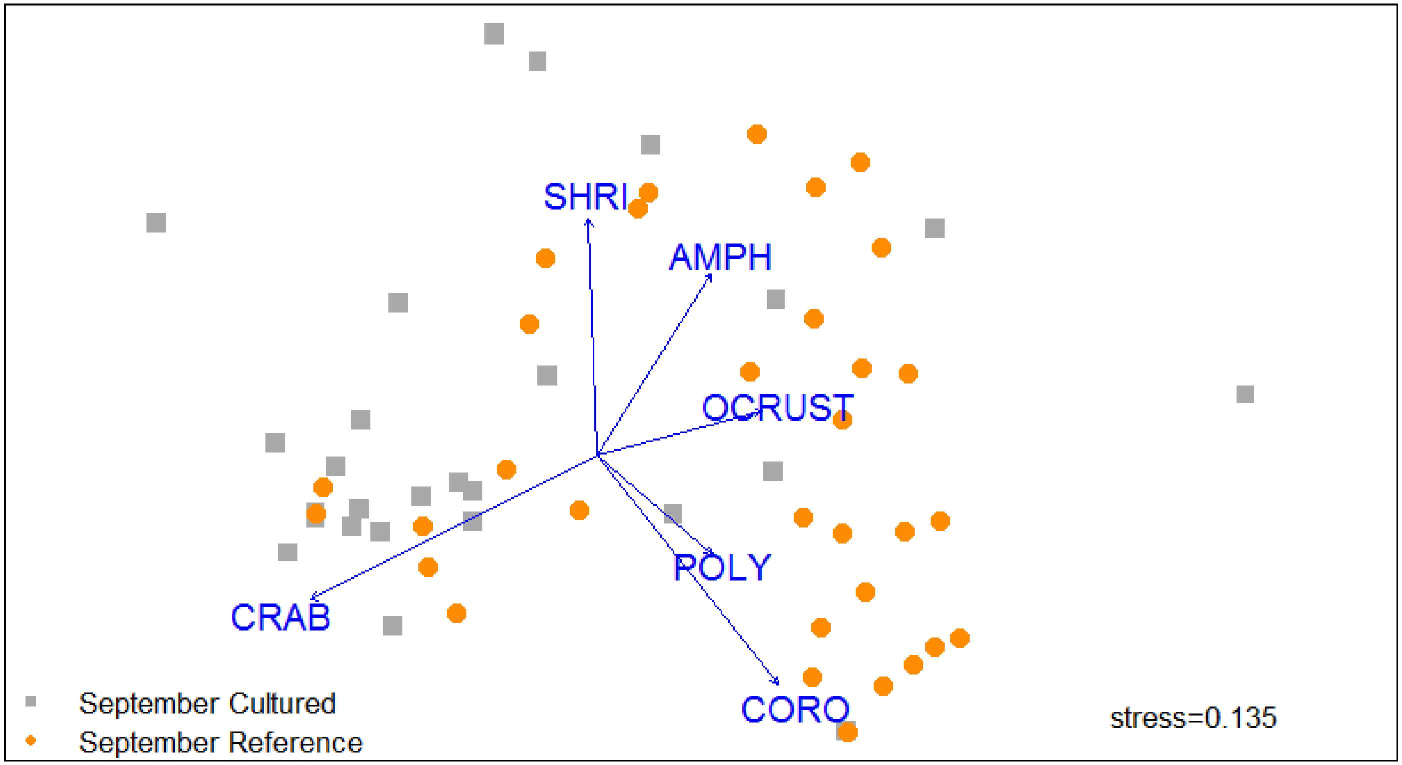

Figure 1.10. NMDS plot of staghorn sculpin stomach contents at cultured and reference plots in September. Significant prey groups ($p<0.05$) overlaid as blue vectors. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp.

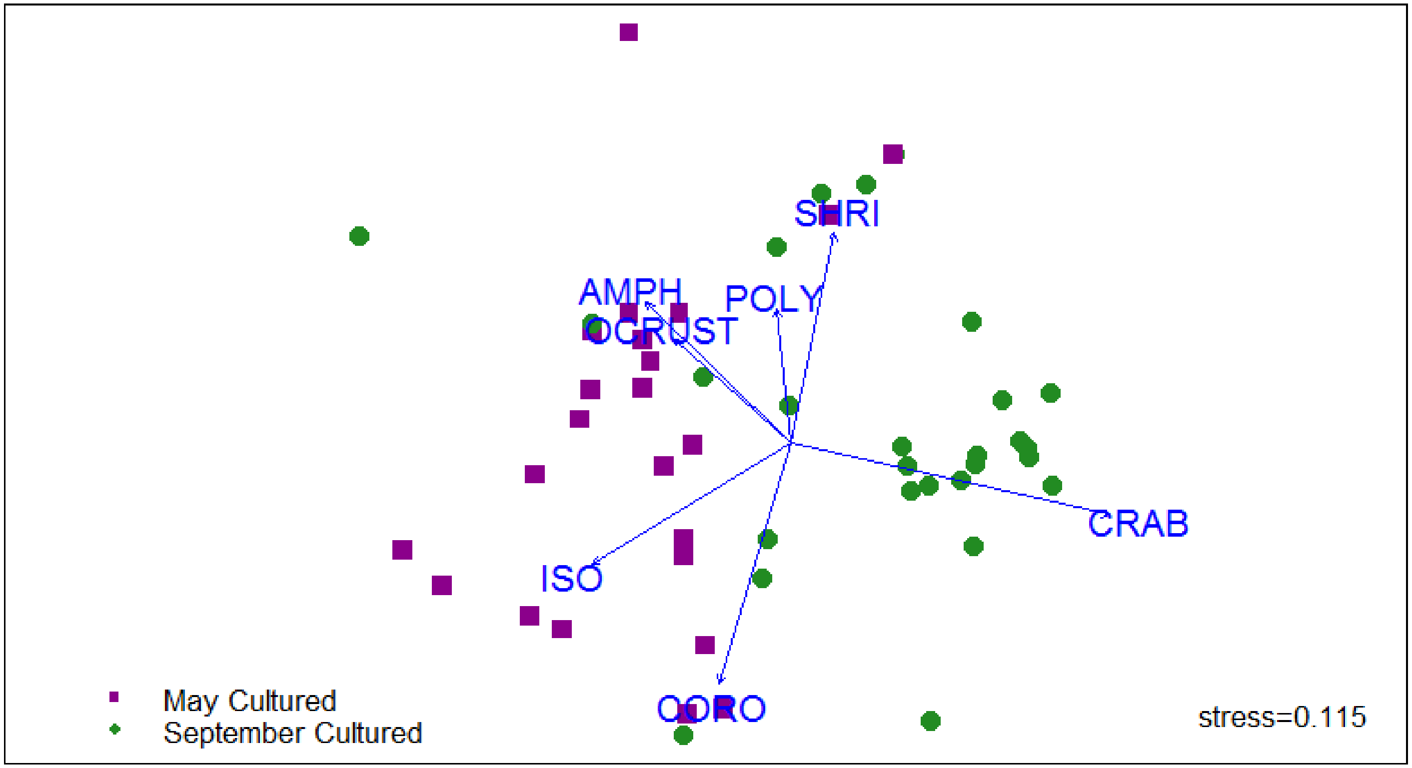

Figure 1.11. NMDS plot of staghorn sculpin stomach contents for cultured plots in May and September. Significant prey groups (p<0.05) overlaid as blue vectors. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp.

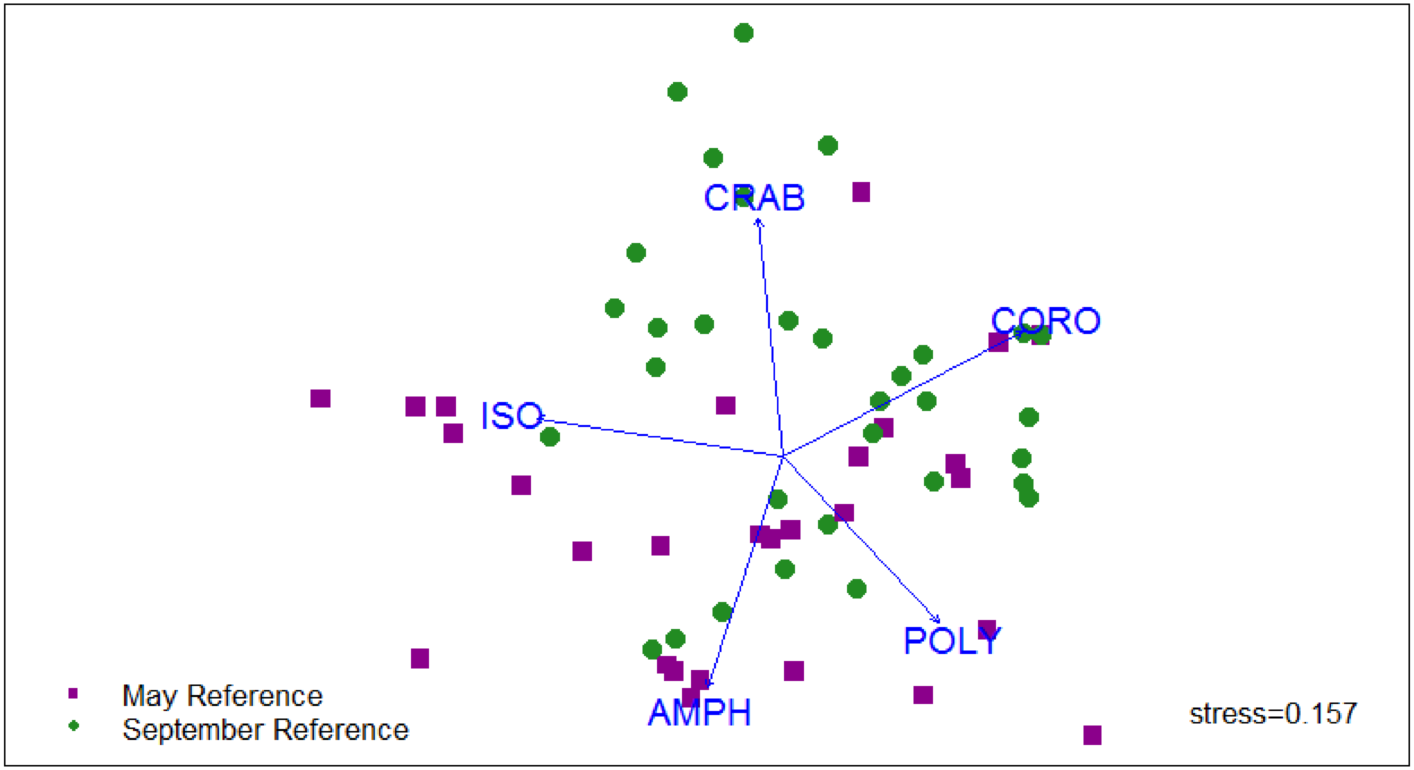

Figure 1.12. NMDS plot of staghorn sculpin stomach contents for reference plots in May and September. Significant prey groups ($p<0.05$) overlaid as blue vectors. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, POLY: polychaetes.

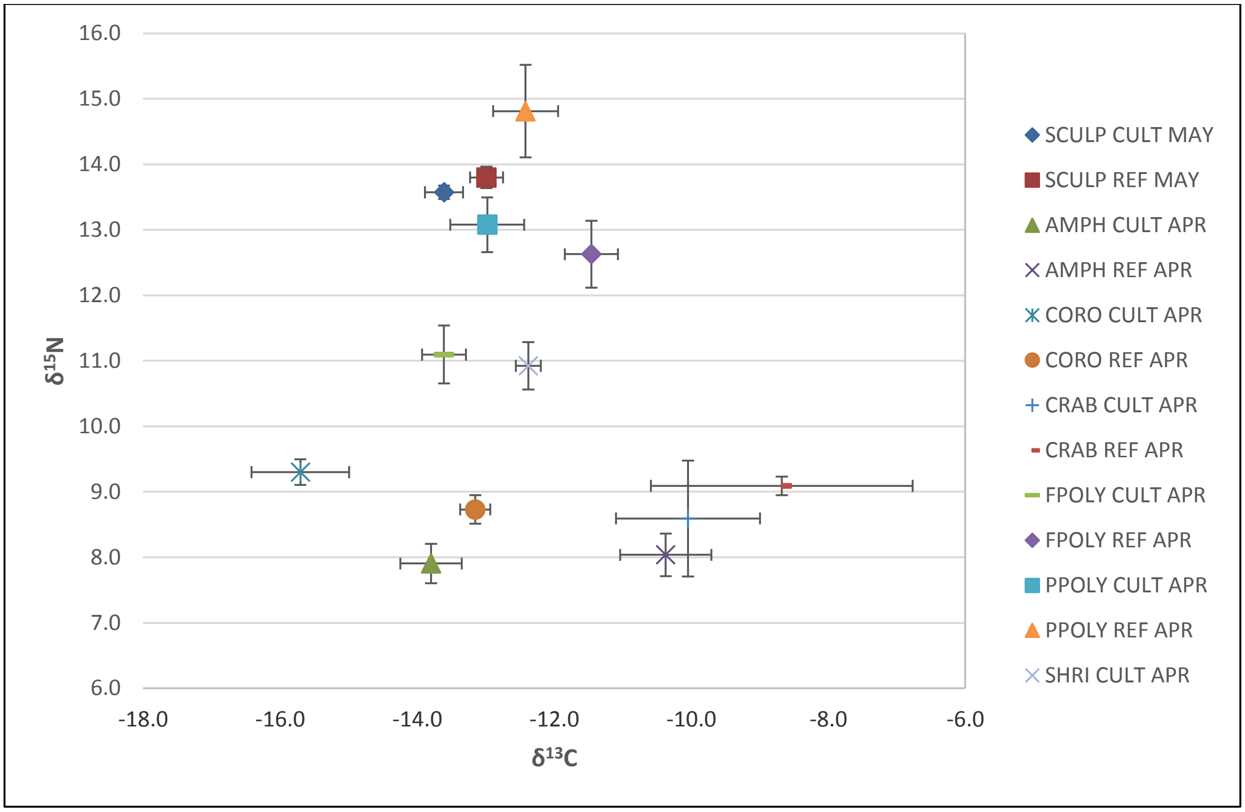

Figure 1.13. Stable isotope biplot of means ±standard error for April prey groups and May staghorn sculpin (SCULP) at cultured and reference plots. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, FPOLY: filter-feeding polychaetes, PPOLY: predatory polychaetes, SHRI: shrimp.

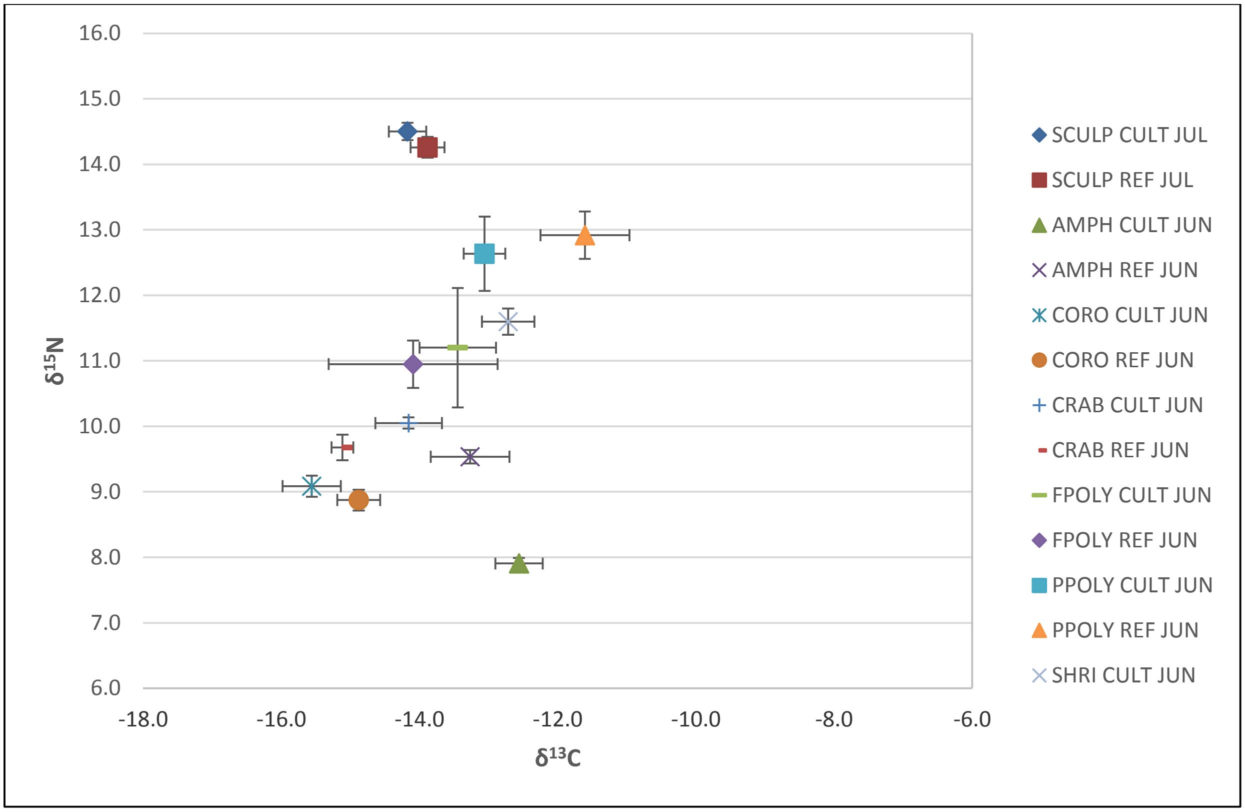

Figure 1.14. Stable isotope biplot of means ±standard error for June prey groups and July staghorn sculpin (SCULP) at cultured and reference plots. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, FPOLY: filter-feeding polychaetes, PPOLY: predatory polychaetes, SHRI: shrimp.

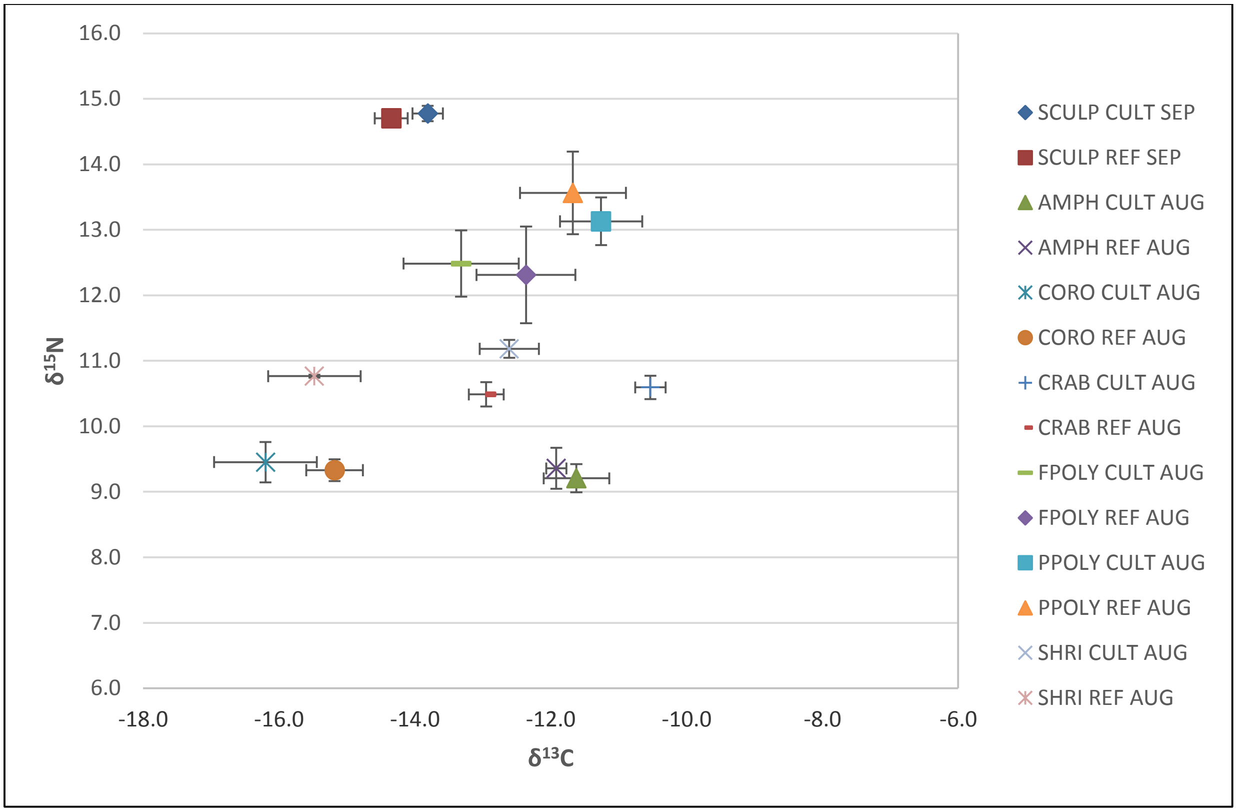

Figure 1.15. Stable isotope biplot of means ±standard error for August prey groups and September staghorn sculpin (SCULP) at cultured and reference plots. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, FPOLY: filter-feeding polychaetes, PPOLY: predatory polychaetes, SHRI: shrimp.

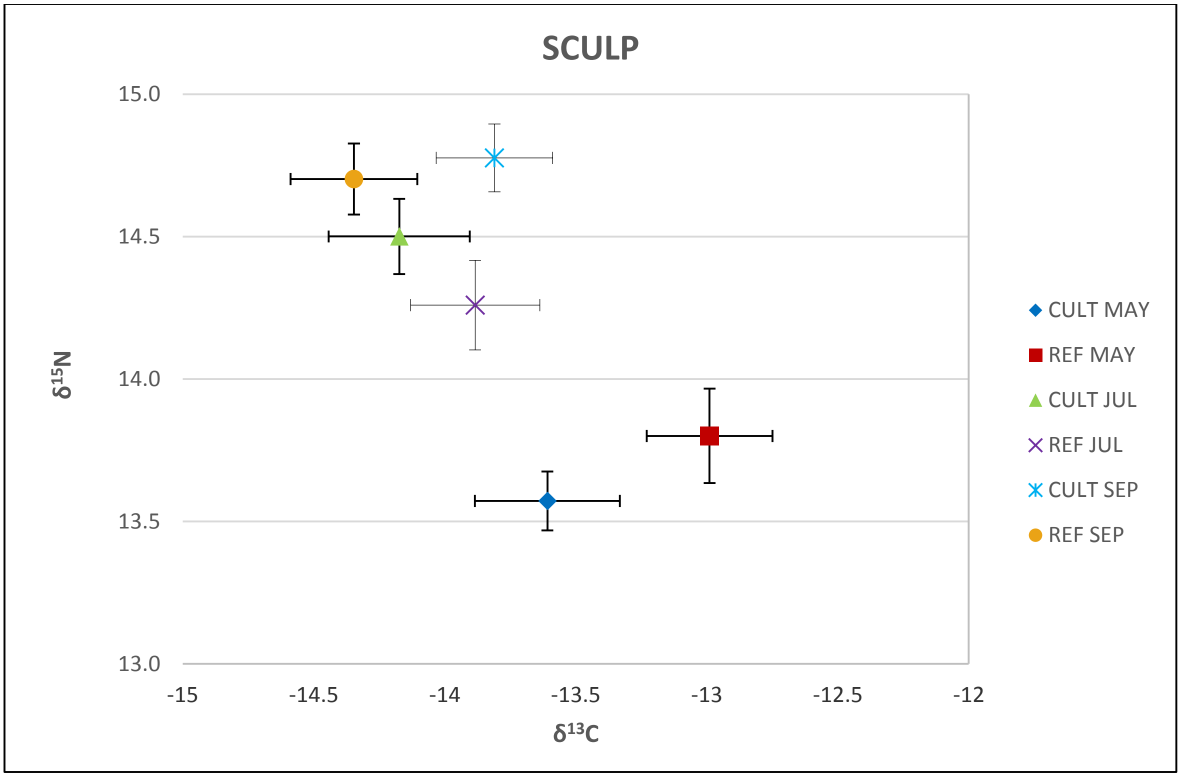

Figure 1.16. Stable isotope biplot of means ±standard error for staghorn sculpin (SCULP) at cultured and reference plots in May, July and September.

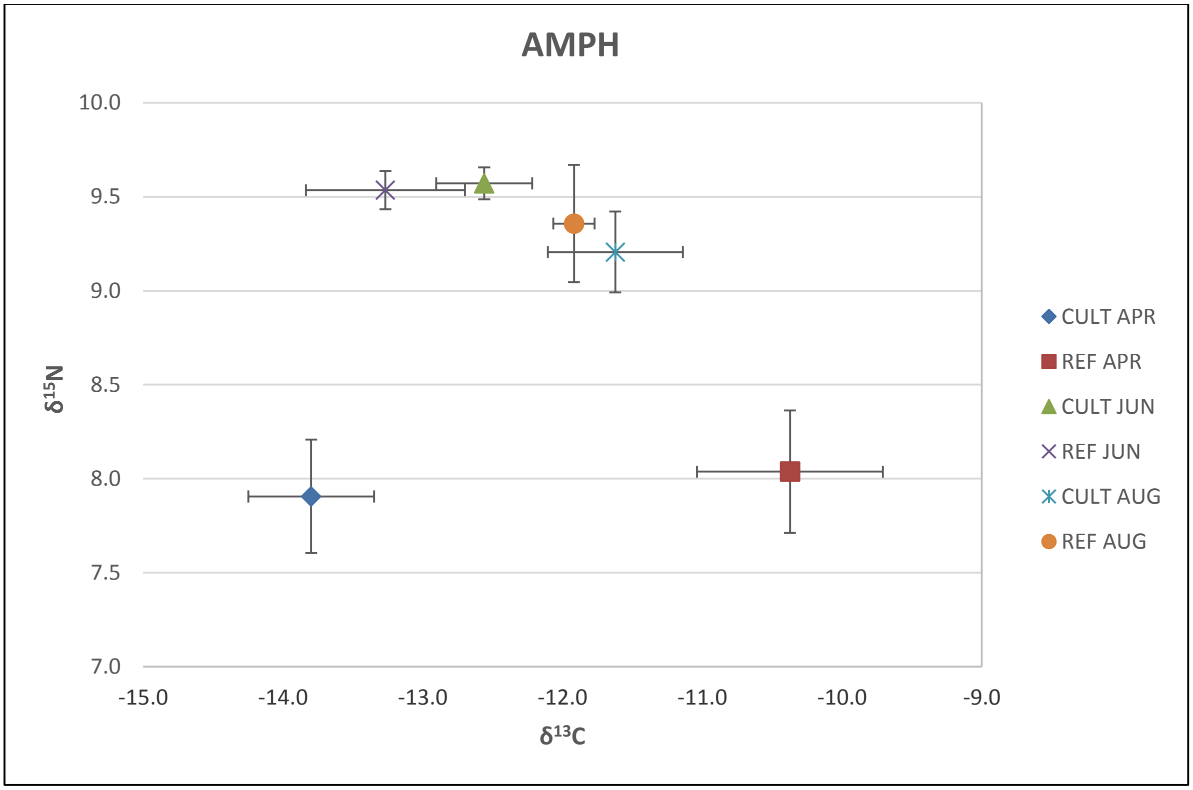

Figure 1.17. Stable isotope biplot of means ±standard error for non-corophium amphipods (AMPH) at cultured and reference plots in April, June and August.

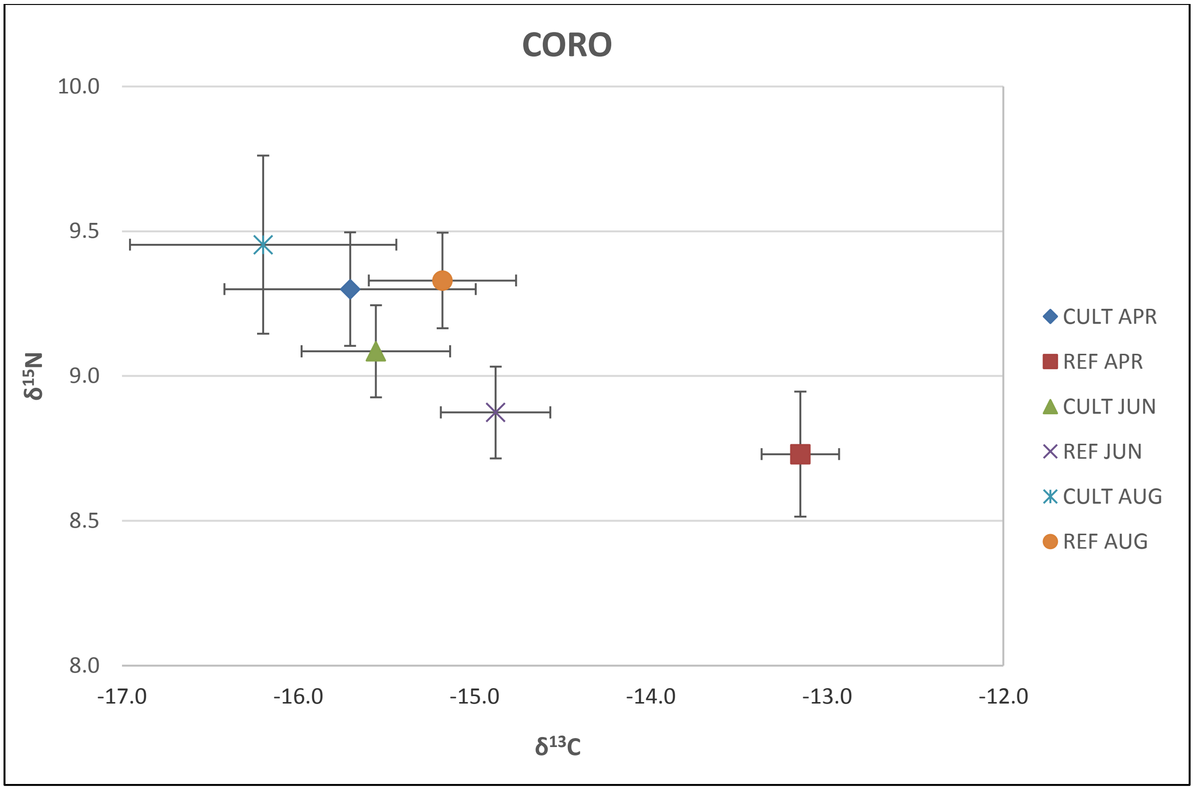

Figure 1.18. Stable isotope biplot of means ±standard error for corophium amphipods (CORO) at cultured and reference plots in April, June and August.

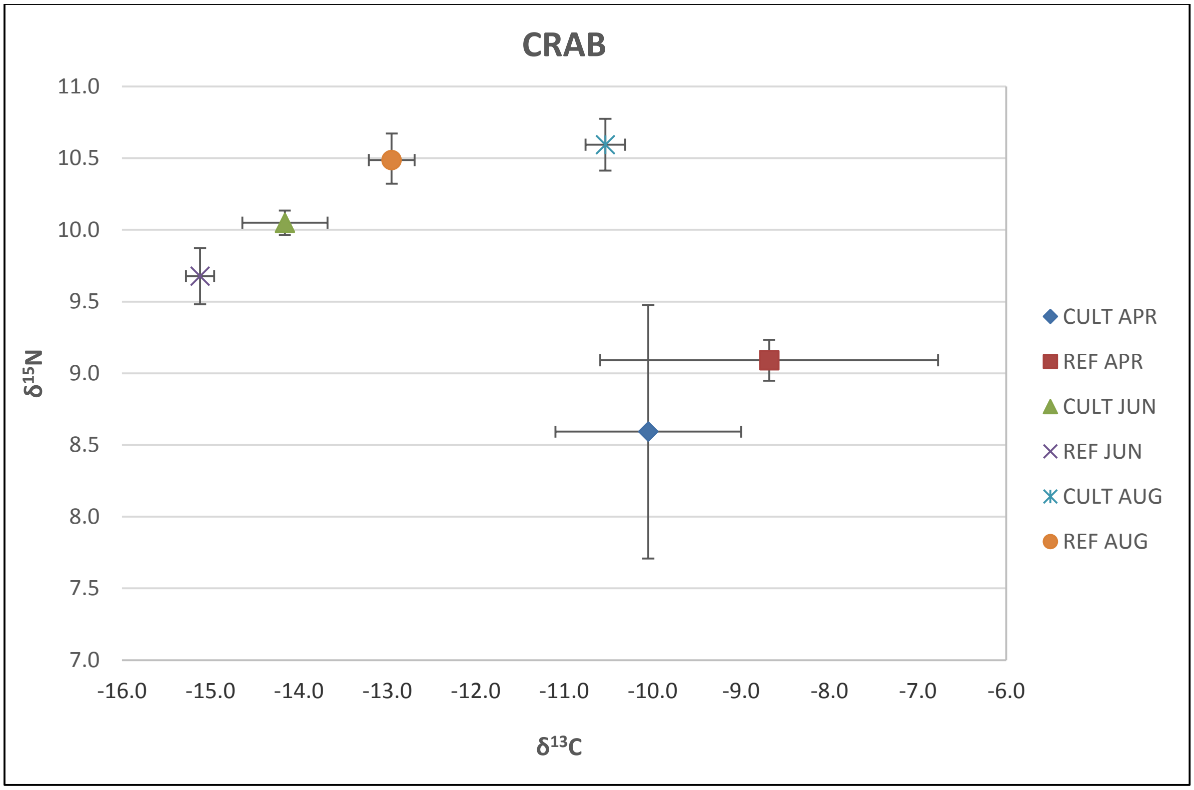

Figure 1.19. Stable isotope biplot of means ±standard error for crabs (CRAB) at cultured and reference plots in April, June and August.

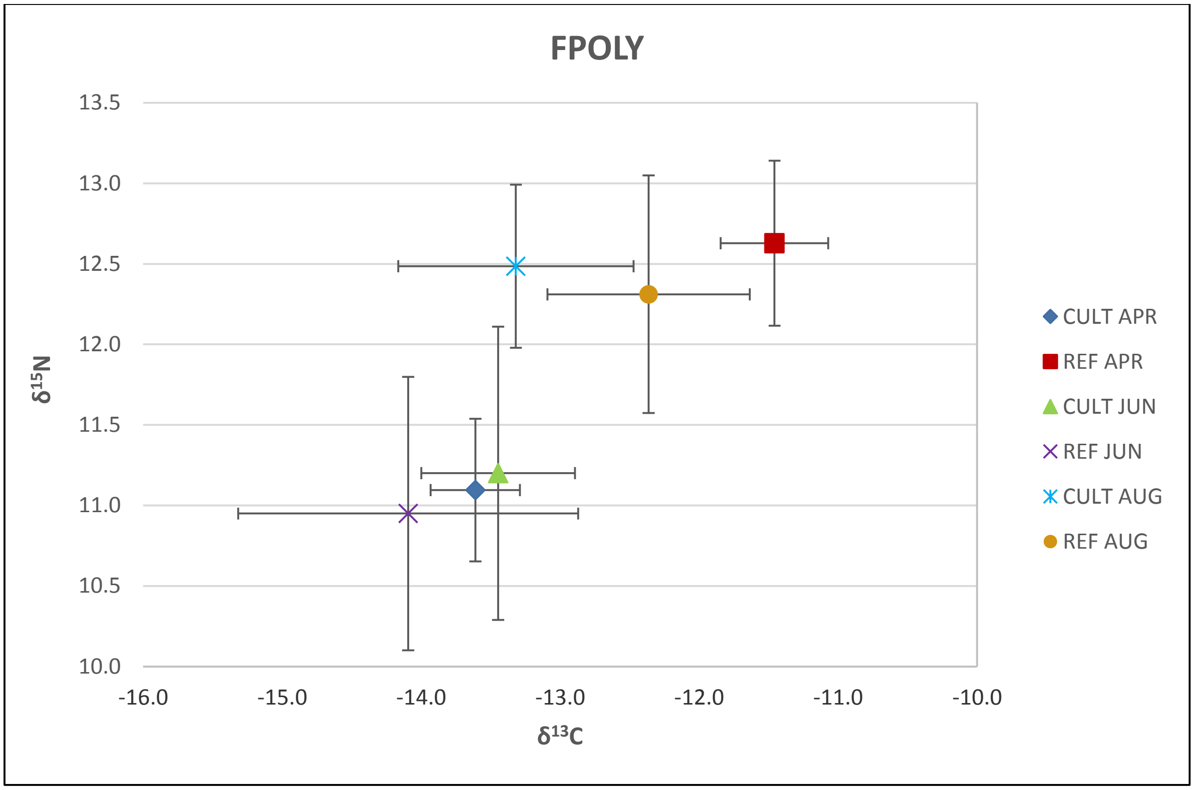

Figure 1.20. Stable isotope biplot of means ±standard error for filter-feeding polychaetes (FPOLY) at cultured and reference plots in April, June and August.

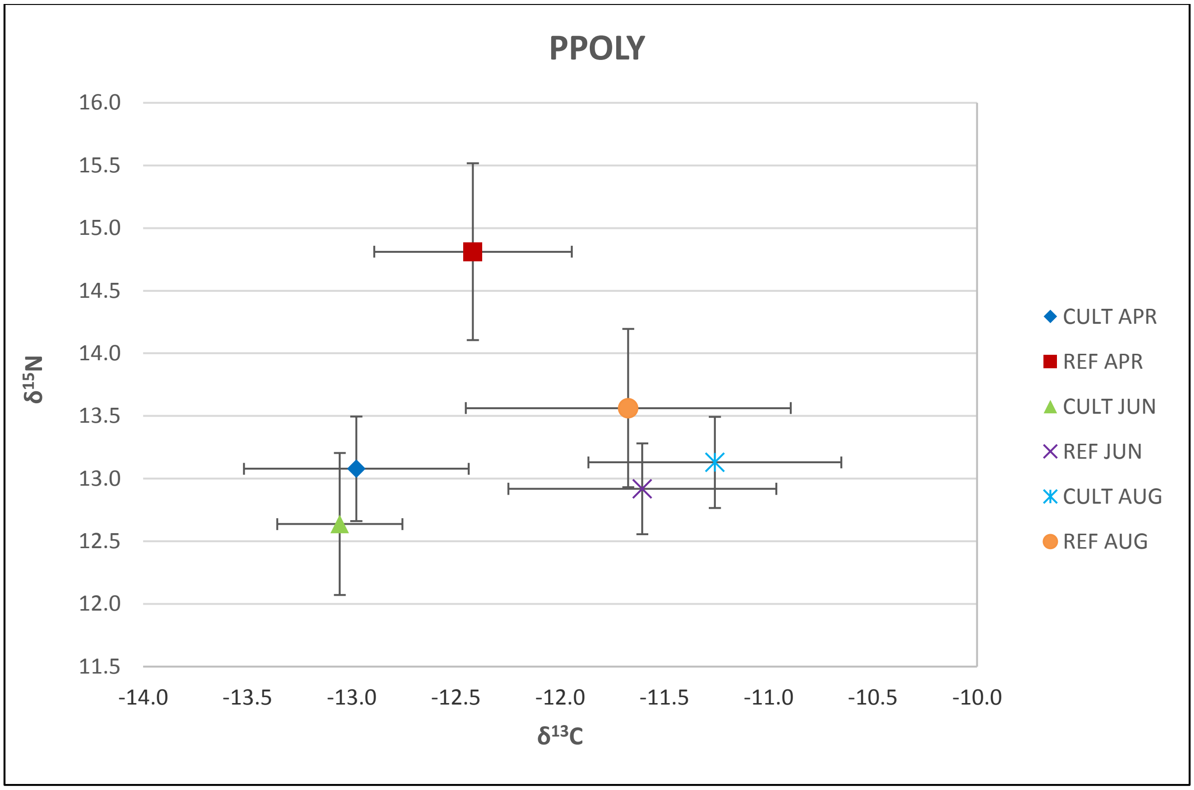

Figure 1.21. Stable isotope biplot of means ±standard error for predatory polychaetes (PPOLY) at cultured and reference plots in April, June and August.

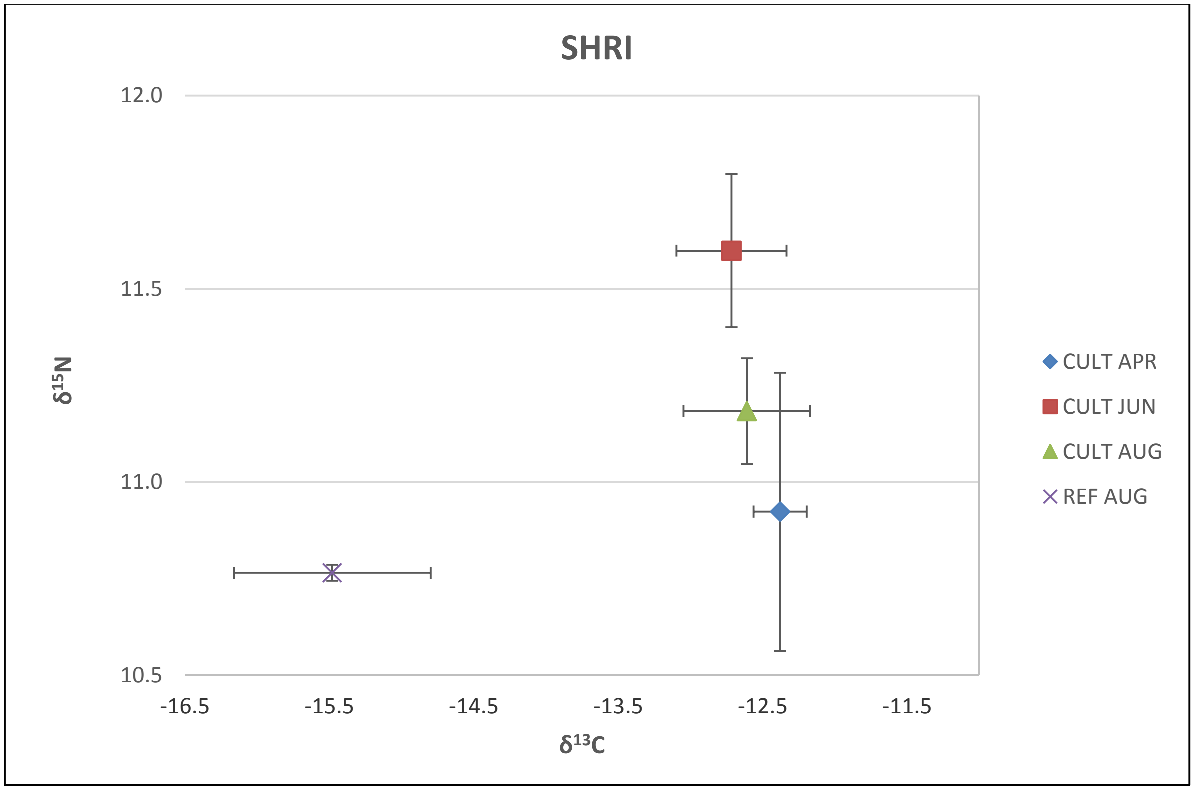

Figure 1.22. Stable isotope biplot of means ±standard error for shrimp (SHRI) at cultured and reference plots (when available) in April, June and August.

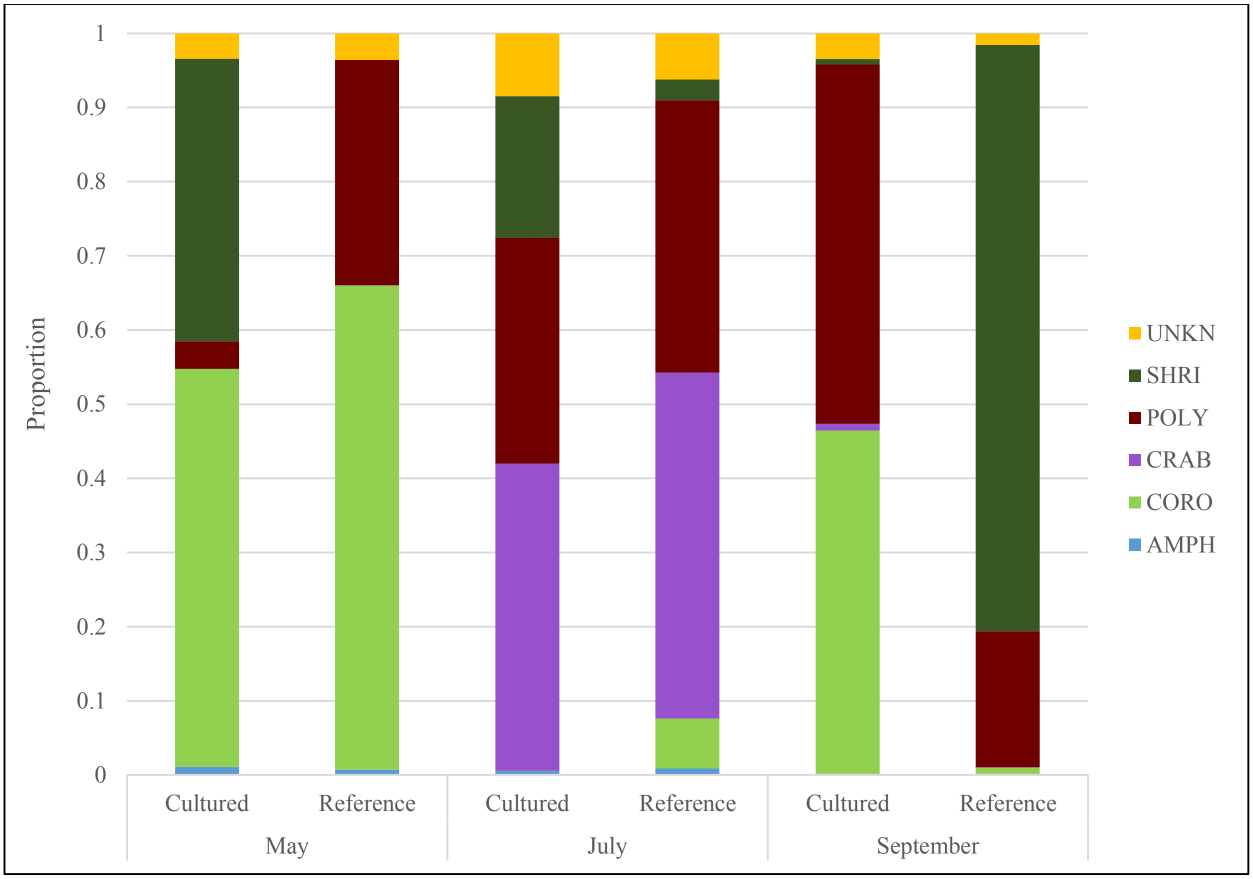

Figure 1.23. Median proportions of each prey group from the SIAR mixing model. AMPH: non-corophium amphipods, CORO: corophium amphipods, CRAB: crabs, POLY: polychaetes, SHRI: shrimp, UNK: unknown.

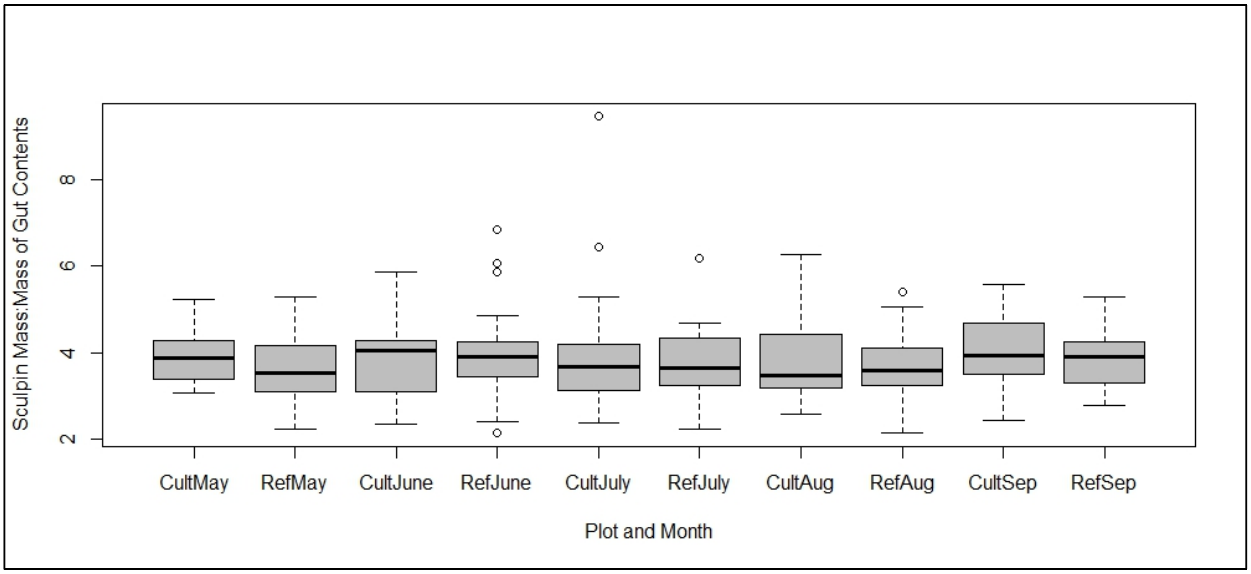

Figure 1.24. Boxplot of the ratio of sculpin mass to the mass of the gut contents for each plot and month.

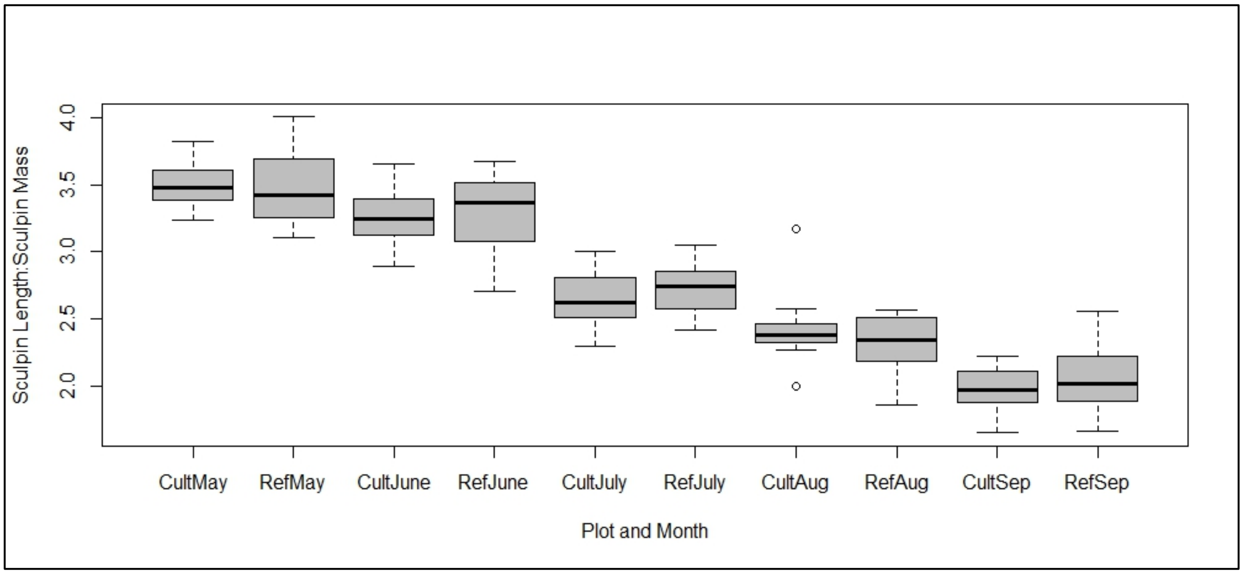

Figure 1.25. Boxplot of the ratio of sculpin length to sculpin mass for each plot and month.

CHAPTER 2

Bioenergetics of Pacific staghorn sculpin (*Leptocottus armatus* Girard, 1854) consumption and growth at geoduck aquaculture farms and nearby reference areas

INTRODUCTION

Decades of research have demonstrated the critical role that ecological disturbance plays in driving community dynamics (Grassle and Sanders 1973, Connell 1978, Sousa 1984, Sousa 2001). Natural disturbance events, such as storms or fire, can have immediate negative impacts on certain species but are often crucial to overall maintenance of an ecosystem (Sousa 1984). Anthropogenic disturbances, such as fishing and aquaculture, can imitate the natural disturbance regime of a particular habitat type, but repeated events may surpass the ability of an ecosystem to recover and retain its reference state (Sousa 1984, Paine et al. 1998). Aquaculture operations are a frequent and prominent cause of anthropogenic disturbance in marine and estuarine communities. As human populations increasingly depend on aquatic food sources for protein and revenue, and populations of wild marine organisms become more depleted, aquaculture is on the rise worldwide (Costa-Pierce 2002). Successful regulation of aquaculture operations is dependent on ecologically oriented research on the effects of disturbances associated with culture activities and the creation of management plans that sustain yields while minimizing environmental impacts.

Aquaculture of Pacific geoduck clams (*Panopea generosa* Gould, 1850; henceforth geoduck) is a valuable industry in Washington State and British Columbia. In Washington State alone, geoduck farming and wild harvests generated an estimated $67 million in gross revenue in 2011 (NOAA 2013). While commercial aquaculture operations boost local economies and increase employment and international trade opportunities, currently there is limited knowledge regarding how geoduck farming alters marine environments (Straus et al. 2009). As operations rapidly expand, management agencies, conservation organizations and community groups have expressed concern regarding potential impacts on local habitat.

Geoduck clams are large, long-lived, burrowing clams found in soft intertidal and subtidal substrata from California to Alaska (Anderson 1971, Shaul and Goodwin 1982, Coan et al. 2000). Intertidal geoduck aquaculture involves high-density placement of nets and polyvinylchloride (PVC) tubes to protect outplanted juveniles from environmental stressors and predators such as crabs and birds (Figure 2.1). Predator-exclusion structures are removed once clams are of a certain size, generally one to two years after planting. The increased size and burrowed depth of clams at 1-2 years of age affords reduced vulnerability to natural predators.

Research on bivalve aquaculture disturbance has shown changes to nutrient and water cycling (e.g. Asmus and Asmus 1991, Giles and Pilditch 2006), sedimentation (e.g. Hayakawa et al. 2001, Hargrave et al. 2008) and biodiversity (e.g. Dealteris et al. 2004, Powers et al. 2007). However, the context and scale of structures used in geoduck aquaculture have not been studied. In 2007 the Washington State Legislature passed Second Substitute House Bill 2220, mandating research on the effects of geoduck aquaculture practices, including evaluation of the ecological consequences of using predator-exclusion structures.

Disturbances from structure additions may cascade through food webs, negatively or positively impacting species at different trophic levels. Research shows enormous variety in the impacts of structures on food web ecology. The addition of aquaculture structures and cultured organisms can increase the diversity and abundance of meiofauna and macrofauna compared to unstructured sites (Dealteris et al. 2004, Powers et al. 2007, Erbland and Ozbay 2008). Algal growth on shellfish aquaculture gear may attract mobile invertebrates and fishes, resulting in greater abundance compared to uncultured, sandy-bottom areas (Powers et al. 2007). Moreover, mobile species may be drawn to structured sites to forage or to take refuge from predators (Guttierez et al. 2003). On the other hand, aquaculture structures might have no effect on the diversity and abundance of mobile species (e.g. Dumbauld et al. 2005, Hosack et al. 2006) or may impact one level of a food web, while other levels remain undisturbed (e.g. Hosack et al. 2006). It is also possible that changes in physical structure may decrease space availability and alter movement patterns of some predators away from farmed regions (e.g. Ribeiro et al. 2007). It seems likely that the ecological impacts of bivalve aquaculture gear will vary greatly depending on the particular farming circumstances, such as the species being cultured and the type and extent of gear used. The diverse outcomes of previous studies on bivalve aquaculture accentuate the need to research the impacts of disparate operations on local and regional scales in order to identify considerations that are applicable to each management planning effort.

Previous studies at geoduck aquaculture sites in South Puget Sound, Washington showed impacts to predator-prey dynamics and biodiversity. Brown and Thuesen (2011) found higher species richness at a geoduck culture site in the structured phase compared to a nearby unstructured control area. In another study, SCUBA surveys indicated that mobile predators common to the study region may exhibit foraging preference for structured or unstructured areas (McDonald et al. in press). For example, flatfishes such as English sole (*Parophrys vetulus* Girard, 1854) were more common in sandy bottom, unstructured areas, while crabs were observed more often at structured sites. Pacific staghorn sculpin (*Leptocottus armatus* Girard, 1854) were seen in high abundance at both habitat types, with preference for unstructured areas in some cases. An examination of the gut contents from staghorn sculpin captured at three cultured plots and their associated unstructured, reference plots in July 2009 showed significant differences between plots in the primary prey groups consumed (Smith and McDonald 2010).

Pacific staghorn sculpin (Scorpaeniformes, Cottidae) are opportunistic, benthic predators common throughout their geographical range from Baja California, Mexico to the Gulf of Alaska (Hart 1973). In parts of South Puget Sound where geoduck farming takes place, staghorn sculpin frequently occur in very high densities (up to one individual per square meter in some cases) and are often the most abundant fish in the community (personal observation). Staghorn sculpin are known to move with the tide into the intertidal zone to feed, and their diet consists of a wide variety of prey, including aquatic invertebrates, such as amphipods and crabs (Tasto 1975). Staghorn sculpin are also vital prey for a variety of birds and fishes (Fitch and Lavenberg 1975) and serve as an important link from lower to higher trophic levels. Because they are opportunistic feeders and occur in a variety of habitat types, staghorn sculpin are useful subjects for studying differences in the available energetic resources at disparate sites. Investigating differences in the consumption of predators such as staghorn sculpin at geoduck aquaculture sites is likely to inform an understanding of the effects of aquaculture operations on food web structure.

A central tool used by ecologists and resource managers to assess energy use by consumers is bioenergetics modeling (Hansen et al. 1993). Bioenergetics models are energy-balance equations that trace the energy from consumption (C) to allocation for metabolism (M), waste (W; includes excretion and defecation/egestion) and somatic and gonadal growth (G) according to the general equation $C=M+W+G$ (Ney 1993). The Wisconsin bioenergetics model (Hanson et al. 1997) is widely used for marine and freshwater fishes. The technique simulates a range of individual processes and responses (e.g. growth and consumption) and has been applied to a variety of aquatic organisms, including fishes (Kitchell et al. 1977, Rice and Cochran 1984, Beauchamp et al. 1989) and invertebrates, such as shrimp and crabs (Rudstam 1989, Chipps and Bennett 2002, Holsman et al. 2003, McDonald et al. 2006). Ecological studies utilize bioenergetics models to provide insights into research topics such as predator-prey interactions (e.g. Walters and Kitchell 2001), ontogeny (e.g. Biro et al. 2005, Beauchamp 2009), habitat use (e.g. Brandt et al. 1992; Stockwell and Johnson 1999) and climate change (e.g. Peterson and Kitchell 2001). To estimate consumption, the model iteratively fits a feeding rate (P-value) that is a proportion of the theoretical maximum daily consumption rate. The P-value is based on the model inputs of temperature, diet composition and growth. The model has been used for a variety of applications (Hartman and Kitchell 2008), such as examining the seasonal consumption demands of salmonids (Baldwin et al. 2000; Armstrong and Schindler 2011) and investigating the foraging behavior of Dungeness crabs (Holsman et al. 2006).

The study objectives were to use the Wisconsin bioenergetics model to assess differences in the growth and consumption demands of staghorn sculpin at sites with and without geoduck aquaculture operations. Because of their high densities at farmed and unfarmed areas in the study region, staghorn sculpin were used as an indicator species to investigate the ecological impacts of the structured phase of geoduck aquaculture. The following questions were posed:

1. Do staghorn sculpin growth and consumption differ between farmed and unfarmed habitats?
2. What are the growth and consumption rates of different sculpin size classes?
3. Does one habitat type provide an energetic advantage to sculpin over the other?
4. How many staghorn sculpin are supported by each habitat?

To address these questions, stomach content analysis, mark-recapture and the Wisconsin bioenergetics model were used to estimate consumption and growth of two sizes classes of staghorn sculpin at habitats with and without geoduck aquaculture. Population consumption was compared to biomass of important prey groups to estimate the carrying capacity of staghorn sculpin at both habitat types.

MATERIALS AND METHODS

Study Area and Design

All study sites are located in Case Inlet in southern Puget Sound, Washington State. Each site was leased by Taylor Shellfish Farms, Inc. (Shelton, WA) for the purpose of farming geoduck clams. The sites used were “Foss” in 2011 and Foss, “Manke” and “Rolfs” in 2012. Foss (N47°13.447’ W-122°49.126’) is located on the eastern shore of Case Inlet near Joemma Beach State Park. Manke (N47°12.104’ W-122°50.263’) and Rolfs (N47°14.329’ W-122°51.548’) are situated on the western shore of Case Inlet, on Harstine Island (Table 2.1, Figure 2.2). All sites consisted of a farmed/structured “cultured” plot and an associated unfarmed/unstructured “reference” plot, each with an area of 2500 m^2. Cultured plots occurred on active geoduck farms in the structured phase of the farming cycle, with cover nets and tubes in place for the duration of the study. Reference plots were areas without aquaculture and no history of geoduck aquaculture, located at least 100 meters from each cultured plot. Plot shape, tidal height and general resident species composition were similar between plots prior to aquaculture operations. However, benthic assemblages at the sites were by no means identical, as noted in McDonald et al. (in press) and VanBlaricom et al. (in press). Both studies documented spatial and temporal variability in the intertidal benthic assemblages of Case Inlet.

Model Parameterization

R (R Development Core Team, version 2.14.2) was utilized for the modeling process and all data analysis. In the Wisconsin bioenergetics model, energy consumed is balanced by energy used for growth and metabolism, as well as energy lost to waste (excretion and egestion). Growth includes somatic and gonadal growth. Metabolism encompasses respiration (routine metabolism, including basal metabolism and standard activity) and energy for food assimilation (specific dynamic action). Respiration is based on water temperature, fish mass and an activity constant (Stewart et al. 1983, Hanson et al. 1997). Activity constants can range from zero to over ten, depending on the species of fish and whether swimming speed is considered to be a constant or a function of other parameters (Hanson et al. 1997). An activity multiplier of 1.0 was used for staghorn sculpin, which corresponds to a constant swimming speed. Excretion and egestion are proportional to consumption (Kitchell et al. 1977) and consumption is dependent on the mass and thermal experience of the consumer (see Table 2.2 for basic equations, below for detailed equations). The model fits a constant (P-value) that is the proportion of maximum consumption (C_{max}) based on temperature and specific consumption (Hanson et al. 1997).

A consumption equation for cold water fish (Thornton and Lessem 1978, Hanson et al. 1997) was used for staghorn sculpin. The equation is a temperature dependent function as follows:

$$f(T) = ((CK1 \times (e^{(G1 \times (T-CQ))})) / (1 + CK1 \times ((e^{(G1 \times (T-CQ))}) - 1))) \times ((CK4 \times (e^{(G2 \times (CTL-T))})) / (1 + CK4 \times ((e^{(G2 \times (CTL-T))}) - 1))$$

Where:

$G1 = (1 / (CTO - CQ)) \times \ln((0.98 \times (1-CK1)) / (CK1 \times 0.02))$

$G2 = (1 / (CTL - CTM)) \times \ln((0.98 \times (1-CK4)) / (CK4 \times 0.02))$

T is water temperature in degrees Celsius; CQ is the temperature for CK1, the low temperature at which consumption is a relatively small fraction of peak consumption; CK1 is the proportion of C_{max} at CQ; CTO is the temperature for CK2, the temperature at which peak consumption occurs; CTL is the temperature for CK4, the high temperature at which consumption is below peak consumption; CK4 is the proportion of C_{max} at CTL; and CTM is the temperature for CK3, which, for staghorn sculpin, is the same as CK2.

Data entered into the model included: physiological parameters for consumption, respiration, excretion and egestion (Table 2.2); average daily temperature for each site and plot (Table 2.3; table shortened to include mean daily temperature for every seventh day); growth for each individual staghorn sculpin; and diet composition for each site, plot, month and size class of sculpin (Table 2.4). The physiological parameters were borrowed from a model for prickly sculpin (*Cottus asper* Richardson, 1836) in Lake Washington, Washington USA (Moss 2001). A sculpin energy density of 4532 J/g was also borrowed from Moss (2001). The Wisconsin model operates on a daily time step. The modeling period of May through August was defined by the period of peak staghorn sculpin density in the study region. Spawning costs were not taken into consideration in the model, as staghorn sculpin are known to spawn during winter months (Wydoski and Whitney 2003), which were not represented in the modeling period.

Model Application

Temperature/Thermal Experience

Maxim iButton® temperature loggers (product number DS1921Z, Maxim Integrated™, San Jose, California USA) secured in the manufacturer's capsule (product number DS9107, Maxim Integrated, San Jose, California USA) were used at each site and plot to record temperature on an hourly basis throughout the study. Temperature loggers were placed in the middle of the plots, inserted in PVC tubes, held in place with cable ties and replaced monthly. Because intertidal plots are frequently exposed during some portion of the tidal cycle, data from iButtons were sorted to select only periods when the device was submerged. This was accomplished by matching time-stamped temperature records with hourly tide height predictions for McMicken Island in Case Inlet, Washington. Tide height data were accessed online from the Department of Biological Sciences at the University of South Carolina (http://tbone.biol.sc.edu/tide/tideshow.cgi). A quality control check confirmed that all spikes in temperature due to tidal exposure were excluded from the final dataset. A daily average temperature was calculated for each plot and site from the submerged temperature values (Table 2.3; table shortened to include mean daily temperature for every seventh day).

Growth

Pacific staghorn sculpin were collected one day per month at each site with a pole seine (length 8.7 m, height 1.7 m) during ebb tides in July-August 2011 and May-August 2012 at cultured and reference plots. Sculpin over 65 mm total length were tagged, measured to the nearest mm and weighed to the nearest 0.1 g before a recovery period and subsequent release back onto the plot of capture. Uniquely numbered T-bar anchor tags (product number FF-94 super short, Floy® Tag & Manufacturing, Inc., Seattle, Washington USA) were secured with fine fabric tagging guns just below the dorsal fin, into the muscle tissue between the spinal processes. Effectiveness of the tagging procedure (tag retention and mortality, see Results for further detail) was tested in the laboratory prior to beginning fieldwork. Tags and needles were sterilized with providone-iodine solution in the laboratory and with 70% ethanol in the field prior to tagging each fish. Before tagging, staghorn sculpin were anesthetized in a water bath with medical grade carbon dioxide from a compressed gas cylinder. Recaptured sculpin were weighed, measured and released to the plot of most recent capture. For each seining event, the total catch of sculpin was noted for each plot and a random subset of the staghorn sculpin less than 65 mm (at least 50 individuals/plot, if available) was measured before release to the plot of capture.

Diet Composition

Stomach contents were analyzed monthly for at least eight non-empty staghorn sculpin caught on ebb tides at each site and plot from May through August, 2012. Because the guts of staghorn sculpin caught during flood tides in earlier studies were mostly empty (R. Smith and P.S. McDonald, unpublished data), the study assumed that sculpin moved in with the tide to feed at cultured or reference plots, remaining until the tide receded. Thus, sculpin gut contents were assumed to be from a feeding episode that occurred at the location where the individual was caught, in the few hours immediately preceding capture (see also results section for supporting mark-recapture data).

A random subset of fish captured during each seining event was euthanized in a buffered water bath with MS-222. Tissues were fixed in buffered 10% formalin and transferred to 70% isopropanol after 24-72 hours. Prior to stomach extraction, each fish was blotted dry, weighed and measured. Gut content analysis included weighing the full and empty stomach, estimating the percent fullness of each stomach and identifying, enumerating and weighing the contents to the lowest possible taxonomic level. All weights were blotted dry wet weights to the nearest 0.0001 g.

Stomach content data were computed as the proportional weight contribution of each prey type to the total mass of stomach contents for each size class of sculpin per month, site and habitat type (Table 2.4). The diet proportions used in the model were constant for each month, such that all model days within a month for a particular site, plot and sculpin size class drew from the same diet information. Guts that were empty or contained only unidentifiable material were excluded from the dataset. Prey groups were created based on broad taxonomic group and frequency of occurrence, resulting in the following eight categories: amphipods excluding *Americorophium salmonis* (Stimpson, 1857) and *Monocorophium* spp. (AMPH), bivalves (BIV), the amphipod species *Americorophium salmonis* and *Monocorophium* spp. (CORO), crabs (CRAB), isopods (ISO), other crustaceans including ostracods, cumaceans and tanaidaceans (OCRUST), polychaetes (POLY) and shrimp (SHRI). Prey energy densities (Table 2.5) were taken from the literature and used to calculate an energy value for the diet of sculpin at each site, plot and month (Table 2.4). When possible, energy densities from local (Puget Sound region) studies were used.

Feeding Rates and Individual Growth and Consumption

The model was initially run for 54 staghorn sculpin recaptures for which specific growth data were available. The 54 individuals were chosen based on the time interval between tagging and recapture (June to early September) and because they represented both years (2011 and 2012), plot types (cultured and reference) and size classes (<100 mm and ≥100 mm). Size classes were determined from scatter plots of sculpin length compared to the relative mass contribution of the five most common prey groups (AMPH, CORO, CRAB, ISO and SHRI) at cultured and references plots for each site (Figures 2.3a-2.3e). The scatter plots indicate a general shift in diet for several prey groups at fish lengths of ~100 mm, so this length was used to demarcate the two size classes. Diet composition for every site was averaged for each plot, month and size class to create the proportions of each prey group that were entered into the model.

Feeding rates (P-values) generated for the 54 sculpin recaptures were averaged for each year, site and plot combination. The remaining recaptured fish (N=17) were not included in the recapture subset to generate P-values because they occurred during months with insufficient data to be included in the modeling period. Mean P-values from the sculpin recaptures were used to project the model forward two weeks from capture dates and within each sampling month of May through August. Projections were for sculpin captured during each modeled month for which no growth data were available (fish measured in the field but not recaptured at a later date) such that sculpin captured in May were modeled for May, sculpin captured in June were modeled for June, etcetera. The two-week modeling period was chosen to conservatively estimate growth and consumption for the month in which each fish was captured, based on the most site and plot specific data available. For sculpin with mass and length data, weight was calculated as a function of length and the resulting regression equation was used to convert lengths of sculpin with unknown weights to a starting weight used in the model projection. Resulting simulations were averaged based on year, site, simulation day, plot and sculpin size class and used to compare total growth, total consumption and growth efficiency (a proportion of specific growth to specific consumption and a metric of grams of growth per gram of food consumed) between plots, across the simulation period for each year and site.

An information-theoretic approach using Akaike's information criterion (AIC; Akaike 1973) was employed to analyze P-values from the recaptured sculpin. Increasingly popular for analysis of ecological data, AIC holds several advantages over traditional hypothesis testing methods (Symonds and Moussalli 2011). Instead of using step-wise comparisons, AIC compares multiple models at once and ranks each model according to goodness of fit with an AIC value. The user can compare models and variables to one another by assessing differences in goodness of fit (Burnham and Anderson 2002, Garamszegi 2011). Akaike's information criterion does not use statistical p-values (not to be confused with P-values that represent feeding rates and are generated by the bioenergetics model) or predetermined thresholds of significance. Instead, the focus is on the reliability or precision of each model.

For recaptured staghorn sculpin, a list of candidate linear models was created for each year based on the main effects of site (S; 2012 only), plot (P) and sculpin length (L) for the response variable of P-value. A modified version of AIC (AICc) that adjusts for small sample sizes was used: $AICc = -2*\ln(L) + 2*K + (2*K*(K+1))/(n-K-1)$, where L is the maximum likelihood estimate for the model, K is the number of parameters including the intercept and n is the number of data points. Delta AICc (ΔAICc) is the difference between each candidate model and the model with the lowest AICc value. Models with ΔAICc values less than 2 are considered to be just as good a fit to the data as the top model (Richards 2005). The strength of each candidate model (i) was compared using an Akaike weight (W_i) and an evidence ratio (ER; the weight of the best model divided by the weight of another candidate model). Akaike weights were calculated from ΔAICc values and represented the probability that a specific model was the model of best fit (Burnham and Anderson 2002).

After generating AICc values, the original list of candidate models was narrowed to generate a 95% confidence set of models with cumulative Akaike weight ≤ 0.95 (Symonds and Moussalli 2011). The resulting models were used to calculate the average weight, standard error and 95% confidence interval for each model parameter (site in 2012, plot, sculpin length and the interaction terms) to assess the effect of each variable on P-value. Categorical parameters were compared to a base model such that Foss was compared to Manke for site (there were no data for Rolfs) and cultured was compared to reference for plot. If zero was included in the confidence interval, the estimate was considered to be no different from zero and thus, it was assumed that there was no effect of the parameter tested (Mazerolle 2006).

Population Consumption

Actual population sizes of sculpin at the study sites are not known, but density measurements obtained from each seine pull provide estimates. While catch numbers of sculpin were higher at the reference plots (Table 2.6), the aquaculture structures likely caused a sampling bias. Sculpin at the cultured plots had more opportunity to escape the seine than at the reference plots. Smaller sculpin at the cultured plots could use the aquaculture nets and tubes as refuge, and fish of any size could escape if the seine was briefly snagged on the aquaculture structures. Due to the potential for sampling bias, only sculpin from specific size ranges represented at both plot types were used in the model projections. Sculpin size ranges were based on total length of all fish captured and designated for each modeled month: May (40-90 mm), June (75-110 mm), July (80-130 mm) and August (80-140 mm).

For each seine pull, the horizontal distance from the point of initiation of the pull to the water's edge (in the direction perpendicular to shoreline), and total number staghorn sculpin caught were recorded. Based on the average sculpin density (0.14 sculpin/m^2 ± 0.04) from May to August at all reference plots in 2012 (Figure 2.4) and a 5000 m^2 plot (the size of a typical geoduck farm), a hypothetical population size of 1000 sculpin per plot was used as a reasonable approximation.

Population-level consumption was based on the average individual consumption output from the Wisconsin model for each year, simulation day, plot and sculpin size class (<100 mm and ≥100 mm). The sculpin year classes were considered to be <1 year (<100 mm) and 1+ year (≥100 mm). Population structure was based on the average proportion of each size class from the seining events. There is little information available in the literature for size-at-age of staghorn sculpin. Generally, along the Pacific coast of the United States, the total length of staghorn sculpin at the end of one year of life varies from 33-132 mm (Wydoski and Whitney 2003). The survival rate of staghorn sculpin is unknown, so rather than using mortality to impose size and age structure on the simulated population, population level consumption was calculated based on the observed frequency of sculpin smaller or larger than 100 mm total length.

Biomass of Prey

Data from benthic cores and epifaunal pump samples were used to compare sculpin population consumption to available biomass of prey at each site. Sediment cores (surface area 0.00196 m^2, depth 10 cm) and epibenthic pump samples (0.0196 m^2) were collected at both habitat types at Foss in July and August 2011 and at all sites in June and August 2012. Prior to sampling, each plot was converted to a Cartesian grid of 100x100 units in Microsoft Excel. A random number generator was used to select ten randomly-positioned points for coring and epibenthic pump sampling without replacement. Epibenthic invertebrates were collected on the ebb tide while water partially covered the plot. Infaunal cores were collected during low tide near the epifaunal sampling locations and from an area undisturbed by the epifaunal sampling process. A PVC tube (diameter 5 cm, length 15 cm) was used for core collection. Epifaunal samplers (Figure 2.5) were based on a design used by Toft and Cordell (2006). During sample collection, the base of the sampler (diameter 15.8 cm) surrounded an area of substrate and a hand pump drew the sample into a 250-micron mesh collection bag attached with a hose clamp to the pump outflow point. Water flowed through the bag, trapping organisms larger than the mesh size. Holes in the sampler base allowed water to enter the device. The holes were covered with

150-micron mesh so no additional epifauna could be sampled from an area other than the enclosed substrate. After 20 pumps, the sample bag was removed and secured in a jar and a clean bag was attached for the next sampling point.

After collection, infaunal and epifaunal samples were preserved in the field with a buffered 10% formalin solution. In the laboratory, both types of samples were sieved through a 500-micron mesh screen and stored in 70% isopropanol until analysis. Benthic organisms were identified at least to family level, enumerated and grouped into the eight prey categories detailed previously. Because the infaunal and epifaunal sampling methods described above only sampled a portion of the total community, prey biomass was calculated for both plot types for all sites and each year. Total counts of each prey group were averaged from infaunal or epifaunal samples (based on life history, whichever sampling method most accurately represents the abundances of each specific prey group), for cultured and reference plots and converted to a total mass based on a 5000 m^2 plot (Tables 2.7-2.10). The average mass of each prey group was calculated from the blotted dry wet weights of at least three replicates of up to 40 individuals stored in 70% isopropanol. Prey group weights were specific to the sampling months of June and August, but mass limitations necessitated combining sites and plots during the weighing procedure. For 2011, biomass calculations for July were based on the average of the prey group weights for June and August. For 2012, biomass calculations for June were used for May, while July biomass was based on the average of biomass for June and August.

RESULTS

Mark-Recapture

Tag retention and mortality associated with the tagging procedure were examined in the laboratory prior to using the method in the field. Twenty-eight randomly selected staghorn sculpin were tagged and held in aquaria for one month. The capture and tagging methods were the same as described in the Methods section. One fish lost its tag (96.4% retention rate) and no sculpin died as a result of the tagging process.

Staghorn sculpin were the most abundant fish captured during beach seining events in all months and in both plot types (Figure 2.6). On average from May through August, 74% of the total catch on cultured plots and 79% of the total catch on reference plots were staghorn sculpin. From 2011-2012, a total of 2681 sculpin were tagged at all sites and plots (Table 2.11). Recapture rates were slightly higher on the reference plots and ranged from 1.0-3.6%, while rates on the cultured plots were 0.0-2.7%. The consistently low percentage of recaptures did not allow for calculation of disappearance rates of staghorn sculpin. Schnabel estimates (Schnabel 1938) for the reference plots suggested population sizes of 2568 (Foss), 5390 (Manke) and 10453 (Rolfs), however the estimates could be biased high due to the low recapture rate. The Rolfs site had the lowest percentage of recaptures and thus, the highest population size estimate. There were 71 total sculpin recaptures, of which 70 were caught on the same plot as originally captured. The results were consistent with site fidelity, and it was concluded that staghorn sculpin exhibited fidelity to cultured or reference plots at the study sites and did not move between plots to forage.

Feeding Rates and Individual Growth and Consumption

Feeding rates (P-values) generated for the 54 sculpin recaptures ranged from ~0.7-1.9 (Table 2.12) and were averaged for each year, site and plot (Table 2.13). Data for 2011 were available only for the Foss site. P-values used for the Rolfs site in 2012 were averaged from Foss and Manke.

AIC values for linear models and Akaike parameter weights for the P-values of staghorn sculpin recaptures are summarized in Tables 2.14-2.15 for 2011 (Foss) and Tables 2.16-2.17 for 2012 (Foss and Manke). The top model set, with cumulative Akaike weights of 0.95 or less, was used to calculate parameter weights for site (2012 only), plot, length and applicable interaction terms.

In 2011 at Foss, plot was included in all three top models (Table 2.14), suggesting at least a moderate effect of plot on the feeding rate of recaptured sculpin. The plot only model was the best model, followed by the model for plot x length. There was a 79% probability that the model of best fit was one of the top two models. Feeding rate was higher on the cultured plot (Figure 2.7). However, the parameter weights showed no effect of plot, sculpin length or the interaction term (Table 2.15).

Analysis of the feeding rates for recaptured sculpin in 2012 showed an effect of site (Table 2.16). There were 18 candidate models and nine models in the top subset with cumulative Akaike weights <0.95. Site was included in all of the top models. The best model was the site only model, with a 43% chance of being the model of best fit. Plot was in over half of the top models, suggesting some effect of plot on feeding rate. However, the model-averaged parameter weights did not indicate an effect of plot. Site was the only parameter to show a strong effect on feeding rate for 2012 (Table 2.17), with higher P-values at Manke on both plots (Figure 2.8).

Thus, geoduck aquaculture initiated some changes in the feeding rates of sculpin but the differences between cultured and reference plots were generally weak.

In July 2011 at Foss, total growth and growth efficiency of smaller (age <1 year) sculpin (<100 mm) were slightly higher on the reference plot and total consumption was higher on the cultured plot (Figures 2.9-2.11). The pattern held for the month of August. The large (age 1+ year) class (≥100 mm) showed a different trend in that growth and consumption were higher on the cultured plot for both months (Figures 2.9 and 2.11). Growth efficiency for large sculpin was also higher on the cultured plot in July, while the 95% confidence intervals overlapped for August (Figure 2.10).

Overall, the 2012 data were more variable, with larger confidence intervals and more overlap between plots (Figures 2.12-2.20). The differences between plots described below were generally minor. Only small sculpin were represented in May at all sites. Very broadly and with a few exceptions, growth and growth efficiency at Foss in 2012 were higher on the reference plot (Figures 2.12-2.13), while Manke and Rolfs showed the opposite pattern (Figures 2.15-2.16, 2.18-2.19). Individual consumption was often similar between plots, especially at Rolfs (Figures 2.14, 2.17, 2.20). Consumption at Foss and Manke generally followed the same trend as growth, with consumption higher on the reference plot at Foss and higher on the cultured plot at Manke.

At Foss in 2012, total growth, growth efficiency and total consumption were higher on the reference plot for the month of May (Figures 2.12-2.14). The months of June, July and August showed a general pattern of negative growth (i.e. mass loss) and growth efficiency on the cultured plot for both sculpin size classes. Both variables were higher on the reference plot or indistinguishable between plots (Figures 2.12-2.13). Total consumption in June through August was similar between plots or higher on the reference plot, depending on the specific month and size class (Figure 2.14).

Unlike Foss, sculpin growth and growth efficiency were positive at both plots for all months at Manke in 2012 (Figures 2.15-2.16). Both response variables were generally higher at the cultured plot for all months and both size classes, with the exception of large sculpin in July. Total consumption was also higher on the cultured plot for both size classes, with some incidences of overlapping confidence intervals.

The bioenergetics projections for Rolfs in 2012 were more variable than the other two sites, with more similarities between plots and size classes within plots (Figures 2.18-2.20). Total growth and growth efficiency were negative for the large fish at the cultured plot in June and for large fish at the reference plot in July. Growth and growth efficiency were either similar between plots or higher on the cultured plot for both size classes (Figures 2.18-2.19). Overlapping confidence intervals did not allow for plot differentiation in total consumption, except in May when consumption was higher on the cultured plot (Figure 2.20).

Generally, growth, growth efficiency and consumption were higher at Manke than at Foss in either year or at Rolfs. The Foss site in 2011 also provided high levels of each response variable, which is interesting given that the results for Foss in 2012 showed very different trends, including some negative growth.

Population Consumption and Biomass of Prey

In 2011 at Foss, total consumption for a population of 1000 sculpin was slightly higher on the cultured plot for July and August (Figure 2.21). Biomass of prey based on a 5000 m^2 plot was much higher at the reference plot in July and slightly higher at the cultured plot in August. At Foss in 2012, simulated population consumption was similar between plots, across months

(Figure 2.22). There was slightly higher consumption on the reference plot, except in June when the cultured plot was somewhat higher. There was more prey biomass at Foss in 2012 on the reference plot for all months. Manke and Rolfs in 2012 had the same pattern of marginally higher consumption on the cultured plots and higher prey biomass on the reference plots for May through August (Figures 2.23-2.24).

Both habitat types at all sites had more than enough prey biomass to support the total consumption of a population of 1000 staghorn sculpin (Table 2.18, Figure 2.25). Population consumption was generally less than 25% of the total biomass. There were a few year, site, month and plot combinations in which total population consumption exceeded half of the total available biomass. Consumption was lower at all sites in the month of May, when large sculpin were not present. Based on the biomass estimates, each 5000 m^2 cultured plot could support ~1300 – 12700 sculpin, while the reference plots could support ~2700 – 36400 sculpin, depending on the site and month. Clearly, reference plots were generally capable of supporting larger population sizes.

Crabs and isopods were underrepresented in the biomass estimates (Tables 2.19-2.21). Neither class of organism was collected efficiently through epibenthic pumps or infaunal cores, likely because of size, a tendency to cling to the substrate and possibly other factors. As a result, both prey groups in the sculpin model projections were consumed in larger amounts than were present according to the biomass calculations. In several cases, the amphipod and corophium prey groups were also depleted in the biomass estimates compared to sculpin consumption, suggesting underrepresentation of the amphipod groups in the infaunal cores.

DISCUSSION

Bioenergetics modeling did not show that geoduck aquaculture explicitly altered the growth or consumption of small or large staghorn sculpin. Differences between cultured and reference plots were generally small and resulted in frequent pattern reversal within sites for the two size classes and several of the months modeled. In many cases, growth and consumption were indistinguishable between plots due to overlapping 95% confidence intervals. Overall, the structured phase of geoduck aquaculture initiated minor changes to sculpin bioenergetics.

The Wisconsin bioenergetics model provides robust estimates of fish growth and consumption, however the accuracy of projections is directly dependent on the quality of the physiological parameters entered into the model (Ney 1993). Consumption and respiration parameters are known to have a particularly large impact on model projection accuracy, while the parameters for excretion and egestion have been shown to contribute less to model error (Bartell et al. 1986). Several of the parameters used to model staghorn sculpin growth and consumption were borrowed from a model developed for prickly sculpin (Moss 2001), while field data on temperature, fish size and diet were specific to the study sites and plots. Prickly sculpin were considered to be the best available surrogate for staghorn sculpin bioenergetics parameters. Borrowing physiological parameters from similar species is not necessarily recommended practice in the field of bioenergetics (Ney 1993, Chipps and Wahl 2008) but time and budget constraints make it somewhat common procedure, especially for understudied species (Tetzlaff et al. 2010). Staghorn sculpin and prickly sculpin do not share all aspects of life history, so it is expected that at least some of the physiological parameters were not entirely accurate in representing staghorn sculpin. For instance, feeding rates (P-values) would normally be less than one. For the sculpin recaptures, the average P-values were greater than one.

However, the primary purpose of the present work was to compare relative differences in sculpin bioenergetics between cultured and reference plots and the same physiological parameters for consumption, respiration, egestion and excretion were used throughout the modeling process. Bioenergetics parameters are known to vary between closely related species (Trudel et al. 2004) and even between populations of the same organism (Munch and Conover 2002, Slaughter et al. 2004). It is realistic to assume that some parameters used here may differ between sites and plots, but studying such detailed aspects of sculpin populations would not only require extensive research on staghorn sculpin physiology under a variety of field conditions, but more importantly, was beyond the scope and objectives of the work reported here.

The biggest differences in sculpin bioenergetics were between sites. While the three study sites had similar habitat characteristics (tidal range, sediment size, beach slope, etc.), site differences were not surprising since the model was run with temperature data specific to each site (and plot) and temperature directly impacts metabolism and feeding rate (Kitchell et al. 1977). Analysis of the feeding rates for recaptured sculpin showed an effect of site in 2012. In 2011 and 2012, there was some effect of plot, as indicated by the top candidate models. However, parameter weights showed no effect of plot in either year, indicating that geoduck aquaculture resulted in overall minor changes to sculpin bioenergetics.

When extrapolated to the population level, the weak effect of geoduck aquaculture on sculpin consumption is more obvious. Sculpin population consumption levels were similar between plots, while differences in prey biomass were more apparent. Biomass was higher overall on the reference plots, where larger populations of sculpin were supported. Both the estimates of biomass and population consumption were conservative, so actual biomass was higher and sculpin population consumption would have been lower than presented here. Some sculpin prey groups were underrepresented by the infaunal and epifaunal sampling, and for simplicity, there was no production rate applied to biomass. In addition, preservatives such as alcohol are known to desiccate and reduce lipid mass in treated invertebrates (Howmiller 1972), so actual masses of prey groups were likely higher than those used. No mortality rate was applied to population consumption for the modeled period. As a result, the estimates of carrying capacity represent a minimal number of sculpin supported at each habitat type. The primary purpose of examining relative differences between plots was not impacted, as the same methods were applied across sites and plot types.

Differences in prey biomass between cultured and reference plots were not entirely unexpected. Other studies have shown that the increased habitat complexity associated with bivalve aquaculture can change the abundance of sculpin infaunal and epifaunal prey groups, such as amphipods, polychaetes and crabs (see Dumbauld et al. 2009). McDonald et al. (in press) found differences in the abundances of specific groups of infauna and epifauna at Puget Sound geoduck aquaculture sites in the structured phase. For example, compared to reference areas, the amphipod *Americorphium salmonis* (part of the corophium amphipod sculpin prey group) and several families of polychaete worms were found in lower abundances in the more complex habitat, a finding that generally corroborates the results here. Some prey groups, such as crabs, were more associated with geoduck aquaculture both in the present study and in the McDonald et al. study.

There is a wealth of research available on the impacts of complex habitats on population and community dynamics. In a classic laboratory experiment using two mite species, Huffaker (1958) reported that in more structurally complex habitat, prey consumption was less efficient compared to structurally simple habitats, and as a result, predator-prey interactions were more stable in cases with greater habitat complexity. In aquatic ecosystems, structural complexity is often associated with reduced foraging efficiency and lower competition and predation (Holt 1987, Hixon and Menge 1991). Competition is thought to be reduced in complex habitat because prey are often more difficult to find (Savino and Stein 1982) and individual predators may be less able to dominate resources when more energy is dedicated to foraging (Basquill and Grant 1998). In a laboratory study, three species of fish showed lower prey-capture efficiency in habitats with increased complexity in the form of macrophytes (Diehl 1988). In a field study, less complex reef habitat was associated with a higher success rate of prey capture compared to coral reefs with greater complexity for three species of marine fishes in Australian waters (Beukers and Jones 1997). Competition of zebrafish (*Danio rerio* Hamilton, 1822) was found to be lower in a more complex vegetated habitat compared to habitat with no vegetation (Basquill and Grant 1998). A variety of factors can influence the success of a predator in foraging and finding refuge in complex habitat. For example, the size-suitability of refuge options, inter-species competition for resources and a variety of behavioral characteristics can be important (Almany 2004).

In the case of geoduck aquaculture and staghorn sculpin, the results suggest that structural complexity does not have a strong effect on consumption and growth, however the actual foraging efficiency of sculpin could differ between habitat types. Sculpin may use more energy to forage on cultured plots to grow at similar rates, as indicated by the slightly higher feeding rates of sculpin recaptures on the cultured plot at Foss in 2011. In several cases, the diets of sculpin were of higher energy content at the cultured plots (Table 2.4), which suggests that there could be an energetic trade-off to foraging in the more complex habitat if diet is of higher quality, but growth and the amount of prey consumed remain approximately the same.

While sculpin prey biomass differed between cultured and reference plots, overall sculpin growth and consumption were not dramatically altered, suggesting that geoduck aquaculture initiated changes in some aspects of the food web but the overall function of sculpin population dynamics within the ecosystem remained unchanged. The reference habitat has the ability to support larger populations of sculpin, an outcome that should be investigated further in future studies on the ecological impacts of geoduck aquaculture. Because of the sampling bias associated with capturing sculpin at the cultured plots, it is not possible here to calculate actual population sizes of sculpin at both habitat types. Perhaps the population size limitation could be overcome in future studies by combining beach seining with other sampling techniques, such as trapping and video monitoring.

It is important to note that the present study is based on data from three non-adjacent geoduck aquaculture sites during the course of one farming cycle. The results cannot be extrapolated to forecast the impacts of geoduck aquaculture operations in close proximity or repeated farming activities at the same location (see Dumbauld et al. 2009). If culture operations were to expand considerably, future studies should focus on the effects of geoduck farms occurring in higher density and use of the same farming location for successive culture cycles. The relatively minor changes to sculpin bioenergetics seen here could be magnified under certain expansion scenarios. In such cases, staghorn sculpin populations could be reduced in Puget Sound, which would have important implications for benthic fauna consumed by sculpin and higher trophic level organisms, such as seabirds, that forage on staghorn sculpin.

The scale of geoduck aquaculture necessary to reduce sculpin population sizes cannot be predicted given the design and data from the present study, so it is imperative that ecological research keep pace with the expansion of geoduck aquaculture operations. Like the opportunistic staghorn sculpin, other mobile predators native to Puget Sound exhibit a degree of foraging plasticity. For example, the speckled sanddab (*Citharichthys stigmaeus* Jordan and Gilbert, 1882) can exploit a variety of intertidal and subtidal food sources, as long as prey are visible in sandy-bottom habitat (VanBlaricom 1982). It is possible that staghorn sculpin can also utilize subtidal areas for foraging, an option which may provide some alternative to intertidal feeding if geoduck aquaculture structures in high density were to create less desirable habitat.

Different organisms use varying strategies to adapt to disturbance events, such as changes in habitat complexity. Some species will benefit from the addition of structure, while others will be harmed. Crabs seem to be drawn to complex, aquaculture habitat (e.g. López-Jamar et al. 1984, McDonald et al. in press), while flatfishes are accustomed to soft-bottom areas and will likely avoid structures that hinder preferred foraging habitat and obscure prey, or increase risks of detection by predators. As a result of the “winners” and “losers” outcome, management agencies could be faced with challenging ethical questions in the event of geoduck aquaculture expansion. Should there be focus on benefiting some species while harming others? How does one weigh the importance of one species over another? Does commercial standing give higher rank than ecological significance? If so, how will other trophic levels be impacted and how can we expect changing Puget Sound food webs to impact people? Moving forward, there are vital ecological questions to address, and the importance of balancing human needs with the preservation of nearshore marine ecosystems cannot be overstated.

Table 2.1. Field site descriptions. MLLW: mean lower low water.

Site	Location	Tidal Range	Substrate (Grain size)	Common Biota
Foss	Case Inlet, west of Joemma Beach State Park	+0.61 m to -0.61 m MLLW	Sandy (~500 µm)	horse clam (*Tresus* spp.), cockle (*Clinocardium nutalli*), graceful crab (*Metacarcinus gracilis*), northern kelp crab (*Pugettia producta*), and Pacific sand dollar (*Dendraster excentricus*)
Manke	Case Inlet, south of Wilson Point on Harstine Island	+0.61 m to -0.61 m MLLW	Sandy with some fines (~250-500 µm)	
Rolfs	Case Inlet, north of Wilson Point on Harstine Island	+0.76 m to -0.61 m MLLW	Sandy with some fines (~250-500 µm)	

Table 2.2. Physiological parameters (Moss 2001) and equations (Hanson 1997) used in the Wisconsin bioenergetics model for Pacific staghorn sculpin. CK1 and CK4 represent low and high temperatures, respectively, at which consumption is lower than peak consumption. CK2 and CK3 are the temperatures at which peak consumption occurs. C_{max}: maximum consumption, p: proportion of maximum consumption, $f(T)$: function dependent on temperature (T), W: sculpin mass.

Model Function	Basic Equation	Description	Value
Consumption (C)	$C = C_{max} \times p \times f(T)$ $C_{max} = CA \times W^{CB}$		
CA		Intercept: Cmax (g/g/d)	0.2325
CB		Coefficient: Cmax versus weight	-0.514
CQ		Temperature for CK1 (°C)	6
CTO		Temperature for CK2 (°C)	18
CTM		Temperature for CK3 (°C)	18
CTL		Temperature for CK4 (°C)	24
CK1		Proportion of peak Cmax at CQ	0.1075
CK4		Proportion of peak Cmax at CTL	0.8129
ACT		Activity multiplier	1
Egestion (F)	$F = FA \times C$		
FA		Constant proportion of consumption	0.16
Excretion (U)	$U = UA \times (C - F)$		
UA		Constant proportion of assimilated energy	0.1

Table 2.3. Mean daily temperatures at each site and plot in 2011 and 2012. Table includes values for every seventh day for each month.

Day	Month	Year	Foss CULT	Foss REF	Manke CULT	Manke REF	Rolfs CULT	Rolfs REF
18	July	2011	13.000	12.875	NA	NA	NA	NA
25	July	2011	13.938	13.688	NA	NA	NA	NA
31	July	2011	13.694	13.463	NA	NA	NA	NA
1	Aug	2011	13.375	13.050	NA	NA	NA	NA
8	Aug	2011	13.987	13.663	NA	NA	NA	NA
15	Aug	2011	13.952	13.220	NA	NA	NA	NA
22	Aug	2011	14.833	14.392	NA	NA	NA	NA
29	Aug	2011	14.119	13.506	NA	NA	NA	NA
1	May	2012	9.703	9.151	8.750	9.021	9.010	9.427
8	May	2012	9.789	9.257	9.704	10.046	10.250	10.217
15	May	2012	11.224	10.266	10.510	10.729	11.172	11.594
22	May	2012	10.269	10.463	10.294	10.225	10.494	11.088
29	May	2012	11.089	11.078	11.349	11.094	11.318	10.953
1	June	2012	10.713	10.781	11.206	10.981	11.563	11.319
8	June	2012	10.838	10.819	10.868	10.800	11.105	10.906
15	June	2012	12.179	11.476	12.113	11.815	12.964	11.220
22	June	2012	11.954	11.842	11.592	12.014	12.822	13.928
29	June	2012	11.900	11.789	12.131	12.572	12.644	12.906
1	July	2012	12.263	12.132	11.711	12.197	12.533	12.289
8	July	2012	12.869	12.523	12.403	12.767	13.435	13.438
15	July	2012	13.394	13.225	12.656	13.013	13.019	13.750
22	July	2012	13.438	12.713	13.019	13.150	12.988	13.694
29	July	2012	13.592	12.789	13.592	13.678	13.632	13.517
1	Aug	2012	13.856	12.888	13.581	13.356	13.694	13.625
8	Aug	2012	13.932	13.521	13.396	13.115	13.802	13.875
15	Aug	2012	14.524	13.726	14.964	14.411	15.869	15.839
22	Aug	2012	14.271	13.816	14.050	13.744	14.716	14.381
29	Aug	2012	14.100	13.538	13.906	13.525	14.256	14.238

Table 2.4. Diet composition as the proportional weight contribution of each prey group from 2012 sculpin stomach contents and diet energy density for each month, site, plot and size class (when available). AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp.

Month	Site	Plot	Size Class	AMPH	BIV	CORO	CRAB	ISO	OCRUST	POLY	SHRI	Energy Density (J/g)
May	Foss	CULT	<100	0.686	0.000	0.082	0.000	0.097	0.003	0.131	0.000	2710.267
May	Foss	REF	<100	0.351	0.000	0.453	0.164	0.000	0.006	0.026	0.000	3235.317
May	Manke	CULT	<100	0.501	0.009	0.234	0.000	0.238	0.004	0.000	0.013	2808.514
May	Manke	REF	<100	0.285	0.000	0.059	0.000	0.000	0.023	0.633	0.001	2311.512
May	Rolfs	CULT	<100	0.553	0.007	0.000	0.000	0.198	0.000	0.000	0.242	3131.312
May	Rolfs	REF	<100	0.223	0.000	0.145	0.000	0.494	0.005	0.133	0.000	2568.677
June	Foss	CULT	<100	0.672	0.000	0.007	0.253	0.023	0.001	0.044	0.000	3298.144
June	Foss	REF	<100	0.821	0.000	0.116	0.000	0.000	0.000	0.063	0.000	2813.245
June	Manke	CULT	<100	0.237	0.002	0.062	0.503	0.065	0.000	0.132	0.000	3706.118
June	Manke	REF	<100	0.053	0.000	0.096	0.416	0.286	0.000	0.150	0.000	3443.461
June	Rolfs	CULT	<100	0.278	0.006	0.008	0.010	0.132	0.027	0.160	0.380	3252.959
June	Rolfs	REF	<100	0.125	0.000	0.010	0.231	0.609	0.022	0.000	0.003	3074.287
July	Foss	CULT	<100	0.019	0.000	0.102	0.000	0.867	0.000	0.000	0.011	2548.388
July	Foss	CULT	≥100	0.022	0.000	0.000	0.650	0.019	0.000	0.291	0.018	3895.826
July	Foss	REF	<100	0.052	0.000	0.666	0.187	0.000	0.000	0.095	0.000	3261.017
July	Manke	CULT	<100	0.238	0.000	0.019	0.167	0.002	0.000	0.000	0.575	4056.964
July	Manke	CULT	≥100	0.011	0.000	0.006	0.468	0.023	0.000	0.064	0.429	4361.605
July	Manke	REF	<100	0.107	0.000	0.625	0.072	0.192	0.000	0.003	0.000	3032.332
July	Manke	REF	≥100	0.000	0.000	0.002	0.998	0.000	0.000	0.000	0.000	4812.832
July	Rolfs	CULT	<100	0.029	0.012	0.006	0.661	0.049	0.111	0.096	0.037	4096.285
July	Rolfs	CULT	≥100	0.000	0.000	0.000	0.971	0.017	0.002	0.000	0.010	4767.551
July	Rolfs	REF	<100	0.172	0.001	0.155	0.012	0.370	0.222	0.061	0.008	2730.542
Aug	Foss	CULT	<100	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2845.000
Aug	Foss	CULT	≥100	0.018	0.000	0.001	0.794	0.187	0.000	0.000	0.000	4337.551
Aug	Foss	REF	<100	0.097	0.000	0.164	0.726	0.001	0.003	0.003	0.007	4315.737
Aug	Foss	REF	≥100	0.024	0.000	0.036	0.940	0.000	0.000	0.000	0.000	4704.456
Aug	Manke	CULT	<100	0.090	0.000	0.005	0.899	0.000	0.000	0.000	0.006	4627.407
Aug	Manke	CULT	≥100	0.013	0.000	0.004	0.959	0.004	0.000	0.000	0.019	4764.355
Aug	Manke	REF	<100	0.809	0.000	0.027	0.136	0.024	0.003	0.000	0.001	3110.821
Aug	Manke	REF	≥100	0.042	0.000	0.004	0.948	0.006	0.000	0.000	0.000	4711.200
Aug	Rolfs	CULT	<100	0.092	0.000	0.009	0.000	0.000	0.019	0.000	0.880	4196.148
Aug	Rolfs	CULT	≥100	0.027	0.000	0.010	0.944	0.000	0.000	0.000	0.020	4737.104
Aug	Rolfs	REF	<100	0.070	0.002	0.416	0.000	0.028	0.035	0.001	0.448	3601.578
Aug	Rolfs	REF	≥100	0.001	0.000	0.004	0.989	0.004	0.001	0.000	0.000	4793.935

Table 2.5. Staghorn sculpin prey groups and energy densities used in the Wisconsin model.

Prey Group	Prey Group Abbreviation	Energy Density (J/g)	Source
Amphipods excluding corophium	AMPH	2845.00	average from Cordell et al. 2011
Bivalves	BIV	1602.47	average from Thayer et al. 1973
Corophium amphipods	CORO	3040.00	Cordell et al. 2011
Crabs	CRAB	4815.78	average from Thayer et al. 1973
Isopods	ISO	2460.00	Gray 2005
Other crustaceans	OCRUST	2914.50	average from Duffy 2003
Polychaetes	POLY	1980.00	Gray 2005
Shrimp	SHRI	4376.46	average from Thayer et al. 1973

Table 2.6. Total numbers of staghorn sculpin captured for combined months of the 2012 field season. (No data for 2011.)

Site	Plot	# Sculpin Captured
Foss	CULT	300
Foss	REF	941
Manke	CULT	711
Manke	REF	1533
Rolfs	CULT	279
Rolfs	REF	1246

Table 2.7. Biomass of sculpin prey groups in 2011 at Foss by month and plot. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp.

Prey Group	**Sample Type**	**Month**	**Site**	**Plot**	**Year**	**Av. Density (#/m^2)**	**Av. Wt. per Indiv. (g)**	**Biomass (g/m^2)**	**Biomass for 5000 m^2 Plot (g)**
AMPH	Infauna	July	Foss	CULT	2011	1732.043	0.0008	1.316	6581.763
AMPH	Infauna	July	Foss	REF	2011	0.000	0.0008	0.000	0.000
AMPH	Infauna	Aug	Foss	CULT	2011	13499.745	0.0012	16.605	83023.434
AMPH	Infauna	Aug	Foss	REF	2011	203.770	0.0012	0.251	1253.184
BIV	Infauna	July	Foss	CULT	2011	2496.179	0.0004	1.028	5137.969
BIV	Infauna	July	Foss	REF	2011	1630.158	0.0004	0.671	3355.408
BIV	Infauna	Aug	Foss	CULT	2011	7896.077	0.0004	3.264	16318.560
BIV	Infauna	Aug	Foss	REF	2011	2496.179	0.0004	1.032	5158.771
CORO	Infauna	July	Foss	CULT	2011	152.827	0.0006	0.085	426.961
CORO	Infauna	July	Foss	REF	2011	55323.484	0.0006	30.912	154559.985
CORO	Infauna	Aug	Foss	CULT	2011	2241.467	0.0004	0.936	4679.063
CORO	Infauna	Aug	Foss	REF	2011	51706.572	0.0004	21.587	107937.468
CRAB	Epifauna	July	Foss	CULT	2011	112.205	0.0067	0.748	3741.096
CRAB	Epifauna	July	Foss	REF	2011	10.200	0.0067	0.068	340.100
CRAB	Epifauna	Aug	Foss	CULT	2011	51.002	0.0125	0.637	3185.937
CRAB	Epifauna	Aug	Foss	REF	2011	20.401	0.0125	0.255	1274.375
ISO	Epifauna	July	Foss	CULT	2011	5.100	0.0005	0.002	11.911
ISO	Epifauna	July	Foss	REF	2011	0.000	0.0005	0.000	0.000
ISO	Epifauna	Aug	Foss	CULT	2011	5.100	0.0002	0.001	4.675
ISO	Epifauna	Aug	Foss	REF	2011	0.000	0.0002	0.000	0.000
OCRUST	Infauna	July	Foss	CULT	2011	7590.423	0.0001	0.899	4496.271
OCRUST	Infauna	July	Foss	REF	2011	4584.819	0.0001	0.543	2715.869
OCRUST	Infauna	Aug	Foss	CULT	2011	20937.341	0.0001	2.681	13405.714
OCRUST	Infauna	Aug	Foss	REF	2011	4737.646	0.0001	0.607	3033.410
POLY	Infauna	July	Foss	CULT	2011	2241.467	0.0006	1.371	6855.154
POLY	Infauna	July	Foss	REF	2011	7080.998	0.0006	4.331	21656.054
POLY	Infauna	Aug	Foss	CULT	2011	6673.459	0.0002	1.474	7368.611
POLY	Infauna	Aug	Foss	REF	2011	10341.314	0.0002	2.284	11418.535
SHRI	Epifauna	July	Foss	CULT	2011	35.702	0.0067	0.238	1190.051
SHRI	Epifauna	July	Foss	REF	2011	5.100	0.0067	0.034	170.007
SHRI	Epifauna	Aug	Foss	CULT	2011	300.913	0.0072	2.162	10807.790
SHRI	Epifauna	Aug	Foss	REF	2011	20.401	0.0072	0.147	732.732

Table 2.8. Biomass of sculpin prey groups in 2012 at Foss by month and plot. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp.

Prey Group	**Sample Type**	**Month**	**Site**	**Plot**	**Year**	**Av. Density (#/m^2)**	**Av. Wt. per Indiv. (g)**	**Biomass (g/m^2)**	**Biomass for 5000 m^2 Plot (g)**
AMPH	Infauna	June	Foss	CULT	2012	815.079	0.0003	0.236	1181.864
AMPH	Infauna	June	Foss	REF	2012	0.000	0.0003	0.000	0.000
AMPH	Infauna	Aug	Foss	CULT	2012	458.482	0.0012	0.564	2819.664
AMPH	Infauna	Aug	Foss	REF	2012	458.482	0.0012	0.564	2819.664
BIV	Infauna	June	Foss	CULT	2012	2750.891	0.0004	1.128	5639.328
BIV	Infauna	June	Foss	REF	2012	254.712	0.0004	0.104	522.160
BIV	Infauna	Aug	Foss	CULT	2012	4941.416	0.0004	2.042	10212.260
BIV	Infauna	Aug	Foss	REF	2012	1375.446	0.0004	0.569	2842.588
CORO	Infauna	June	Foss	CULT	2012	764.137	0.0007	0.535	2674.478
CORO	Infauna	June	Foss	REF	2012	30005.094	0.0007	21.004	105017.830
CORO	Infauna	Aug	Foss	CULT	2012	916.964	0.0004	0.383	1914.162
CORO	Infauna	Aug	Foss	REF	2012	16861.946	0.0004	7.040	35199.312
CRAB	Epifauna	June	Foss	CULT	2012	122.405	0.0008	0.103	516.142
CRAB	Epifauna	June	Foss	REF	2012	10.200	0.0008	0.009	43.012
CRAB	Epifauna	Aug	Foss	CULT	2012	20.401	0.0125	0.255	1274.375
CRAB	Epifauna	Aug	Foss	REF	2012	0.000	0.0125	0.000	0.000
ISO	Epifauna	June	Foss	CULT	2012	10.200	0.0008	0.008	38.294
ISO	Epifauna	June	Foss	REF	2012	5.100	0.0008	0.004	19.147
ISO	Epifauna	Aug	Foss	CULT	2012	0.000	0.0002	0.000	0.000
ISO	Epifauna	Aug	Foss	REF	2012	0.000	0.0002	0.000	0.000
OCRUST	Infauna	June	Foss	CULT	2012	11614.875	0.0001	1.265	6323.654
OCRUST	Infauna	June	Foss	REF	2012	2547.122	0.0001	0.277	1386.766
OCRUST	Infauna	Aug	Foss	CULT	2012	7080.998	0.0001	0.907	4533.806
OCRUST	Infauna	Aug	Foss	REF	2012	4024.452	0.0001	0.515	2576.767
POLY	Infauna	June	Foss	CULT	2012	6265.920	0.0010	6.282	31407.922
POLY	Infauna	June	Foss	REF	2012	16148.752	0.0010	16.189	80945.619
POLY	Infauna	Aug	Foss	CULT	2012	7641.365	0.0002	1.687	8437.341
POLY	Infauna	Aug	Foss	REF	2012	11003.566	0.0002	2.430	12149.771
SHRI	Epifauna	June	Foss	CULT	2012	0.000	0.0062	0.000	0.000
SHRI	Epifauna	June	Foss	REF	2012	10.200	0.0062	0.063	313.663
SHRI	Epifauna	Aug	Foss	CULT	2012	10.200	0.0072	0.073	366.366
SHRI	Epifauna	Aug	Foss	REF	2012	10.200	0.0072	0.073	366.366

Table 2.9. Biomass of sculpin prey groups in 2012 at Manke by month and plot. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp.

Prey Group	**Sample Type**	**Month**	**Site**	**Plot**	**Year**	**Av. Density (#/m^2)**	**Av. Wt. per Indiv. (g)**	**Biomass (g/m^2)**	**Biomass for 5000 m^2 Plot (g)**
AMPH	Infauna	June	Manke	CULT	2012	356.597	0.0003	0.103	517.066
AMPH	Infauna	June	Manke	REF	2012	662.252	0.0003	0.192	960.265
AMPH	Infauna	Aug	Manke	CULT	2012	50.942	0.0012	0.063	313.296
AMPH	Infauna	Aug	Manke	REF	2012	662.252	0.0012	0.815	4072.848
BIV	Infauna	June	Manke	CULT	2012	4024.452	0.0004	1.650	8250.127
BIV	Infauna	June	Manke	REF	2012	1782.985	0.0004	0.731	3655.120
BIV	Infauna	Aug	Manke	CULT	2012	10035.660	0.0004	4.148	20740.363
BIV	Infauna	Aug	Manke	REF	2012	2088.640	0.0004	0.863	4316.522
CORO	Infauna	June	Manke	CULT	2012	2903.719	0.0007	2.033	10163.016
CORO	Infauna	June	Manke	REF	2012	18899.643	0.0007	13.230	66148.752
CORO	Infauna	Aug	Manke	CULT	2012	356.597	0.0004	0.149	744.396
CORO	Infauna	Aug	Manke	REF	2012	12379.012	0.0004	5.168	25841.187
CRAB	Epifauna	June	Manke	CULT	2012	56.102	0.0008	0.047	236.565
CRAB	Epifauna	June	Manke	REF	2012	20.401	0.0008	0.017	86.024
CRAB	Epifauna	Aug	Manke	CULT	2012	20.401	0.0125	0.255	1274.375
CRAB	Epifauna	Aug	Manke	REF	2012	15.301	0.0125	0.191	955.781
ISO	Epifauna	June	Manke	CULT	2012	20.401	0.0008	0.015	76.588
ISO	Epifauna	June	Manke	REF	2012	0.000	0.0008	0.000	0.000
ISO	Epifauna	Aug	Manke	CULT	2012	0.000	0.0002	0.000	0.000
ISO	Epifauna	Aug	Manke	REF	2012	0.000	0.0002	0.000	0.000
OCRUST	Infauna	June	Manke	CULT	2012	17931.737	0.0001	1.953	9762.835
OCRUST	Infauna	June	Manke	REF	2012	10086.602	0.0001	1.098	5491.594
OCRUST	Infauna	Aug	Manke	CULT	2012	5756.495	0.0001	0.737	3685.756
OCRUST	Infauna	Aug	Manke	REF	2012	8863.984	0.0001	1.135	5675.412
POLY	Infauna	June	Manke	CULT	2012	16148.752	0.0010	16.189	80945.619
POLY	Infauna	June	Manke	REF	2012	15537.443	0.0010	15.576	77881.431
POLY	Infauna	Aug	Manke	CULT	2012	14111.055	0.0002	3.116	15580.956
POLY	Infauna	Aug	Manke	REF	2012	16811.004	0.0002	3.712	18562.150
SHRI	Epifauna	June	Manke	CULT	2012	158.107	0.0062	0.972	4861.784
SHRI	Epifauna	June	Manke	REF	2012	5.100	0.0062	0.031	156.832
SHRI	Epifauna	Aug	Manke	CULT	2012	132.606	0.0072	0.953	4762.755
SHRI	Epifauna	Aug	Manke	REF	2012	91.804	0.0072	0.659	3297.292

Table 2.10. Biomass of sculpin prey groups in 2012 at Rolfs by month and plot. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp.

Prey Group	**Sample Type**	**Month**	**Site**	**Plot**	**Year**	**Av. Density (#/m^2)**	**Av. Wt. per Indiv. (g)**	**Biomass (g/m^2)**	**Biomass for 5000 m^2 Plot (g)**
AMPH	Infauna	June	Rolfs	CULT	2012	50.942	0.0003	0.015	73.867
AMPH	Infauna	June	Rolfs	REF	2012	152.827	0.0003	0.044	221.600
AMPH	Infauna	Aug	Rolfs	CULT	2012	152.827	0.0012	0.188	939.888
AMPH	Infauna	Aug	Rolfs	REF	2012	916.964	0.0012	1.128	5639.328
BIV	Infauna	June	Rolfs	CULT	2012	1681.100	0.0004	0.689	3446.256
BIV	Infauna	June	Rolfs	REF	2012	5196.128	0.0004	2.130	10652.063
BIV	Infauna	Aug	Rolfs	CULT	2012	8965.869	0.0004	3.706	18529.462
BIV	Infauna	Aug	Rolfs	REF	2012	23535.405	0.0004	9.728	48639.837
CORO	Infauna	June	Rolfs	CULT	2012	0.000	0.0007	0.000	0.000
CORO	Infauna	June	Rolfs	REF	2012	458.482	0.0007	0.321	1604.687
CORO	Infauna	Aug	Rolfs	CULT	2012	0.000	0.0004	0.000	0.000
CORO	Infauna	Aug	Rolfs	REF	2012	6724.401	0.0004	2.807	14037.188
CRAB	Epifauna	June	Rolfs	CULT	2012	45.902	0.0008	0.039	193.553
CRAB	Epifauna	June	Rolfs	REF	2012	219.309	0.0008	0.185	924.755
CRAB	Epifauna	Aug	Rolfs	CULT	2012	20.401	0.0125	0.255	1274.375
CRAB	Epifauna	Aug	Rolfs	REF	2012	20.401	0.0125	0.255	1274.375
ISO	Epifauna	June	Rolfs	CULT	2012	224.410	0.0008	0.168	842.471
ISO	Epifauna	June	Rolfs	REF	2012	30.601	0.0008	0.023	114.882
ISO	Epifauna	Aug	Rolfs	CULT	2012	5.100	0.0002	0.001	4.675
ISO	Epifauna	Aug	Rolfs	REF	2012	5.100	0.0002	0.001	4.675
OCRUST	Infauna	June	Rolfs	CULT	2012	815.079	0.0001	0.089	443.765
OCRUST	Infauna	June	Rolfs	REF	2012	27967.397	0.0001	3.045	15226.694
OCRUST	Infauna	Aug	Rolfs	CULT	2012	1732.043	0.0001	0.222	1108.989
OCRUST	Infauna	Aug	Rolfs	REF	2012	28120.224	0.0001	3.601	18004.755
POLY	Infauna	June	Rolfs	CULT	2012	5603.668	0.0010	5.618	28088.385
POLY	Infauna	June	Rolfs	REF	2012	6928.171	0.0010	6.945	34727.458
POLY	Infauna	Aug	Rolfs	CULT	2012	30412.634	0.0002	6.716	33580.616
POLY	Infauna	Aug	Rolfs	REF	2012	15282.731	0.0002	3.375	16874.682
SHRI	Epifauna	June	Rolfs	CULT	2012	703.830	0.0062	4.329	21642.781
SHRI	Epifauna	June	Rolfs	REF	2012	5.100	0.0062	0.031	156.832
SHRI	Epifauna	Aug	Rolfs	CULT	2012	438.619	0.0072	3.151	15753.727
SHRI	Epifauna	Aug	Rolfs	REF	2012	183.608	0.0072	1.319	6594.584

Table 2.11. Numbers of captured (all individuals ≥65 mm in length were tagged) and recaptured staghorn sculpin by year, site and plot. * Indicates location of the only individual that was not recaptured on the same plot as original capture.

Site	Plot	Year	# Sculpin Captured/Tagged	# Recaptures	Recapture %
Foss	CULT	2011	296	8	2.7
Foss	REF	2011	290	10	3.4
Foss	CULT	2012	132	2*	0.7
Foss	REF	2012	459	16	3.5
Manke	CULT	2012	395	10	2.5
Manke	REF	2012	581	21	3.6
Rolfs	CULT	2012	116	0	0.0
Rolfs	REF	2012	412	4	1.0
Total			2681	71	

Table 2.12. P-values for sculpin recaptures generated by the Wisconsin model. TL: total length.

Sculpin Number	Site	Plot	Year	Time Interval	Initial TL (mm)	Final TL (mm)	Initial Wt. (g)	Final Wt. (g)	P-value
1	Foss	CULT	2011	July-Aug	95	98	8.2	8.8	1.411
2	Foss	CULT	2011	July-Aug	88	96	7.4	8.9	1.697
3	Foss	CULT	2011	July-Aug	98	108	11.0	13.6	1.385
4	Foss	CULT	2011	Aug-Sep	102	121	11.3	18.8	1.322
5	Foss	CULT	2011	Aug-Sep	98	107	9.1	12.8	1.359
6	Foss	CULT	2011	Aug-Sep	102	118	11.1	18.2	1.293
7	Foss	CULT	2011	Aug-Sep	114	130	15.8	22.8	1.335
8	Foss	REF	2011	July-Aug	95	102	8.4	10.1	1.375
9	Foss	REF	2011	July-Aug	93	97	8.1	9.6	1.317
10	Foss	REF	2011	July-Aug	92	98	7.9	8.9	1.180
11	Foss	REF	2011	July-Aug	80	83	5.7	6.8	1.148
12	Foss	REF	2011	July-Aug	89	93	6.8	7.6	1.091
13	Foss	REF	2011	July-Aug	84	98	8.2	9.5	1.267
14	Foss	REF	2011	July-Aug	101	104	11.9	12.1	1.089
15	Foss	REF	2011	July-Aug	98	104	9.7	11.5	1.402
16	Foss	REF	2011	July-Aug	108	112	11.6	12.9	1.327
17	Foss	REF	2011	Aug-Sep	91	107	8.7	12.4	1.063
18	Foss	CULT	2012	Aug-Sep	99	108	10.7	12.2	0.904
19	Foss	REF	2012	July-Aug	92	95	7.6	8.0	0.858
20	Foss	REF	2012	July-Aug	77	88	4.8	7.0	1.046
21	Foss	REF	2012	July-Aug	86	98	6.6	9.3	1.155
22	Foss	REF	2012	July-Aug	80	91	5.3	7.1	0.995
23	Foss	REF	2012	July-Aug	82	95	5.4	9.0	1.262
24	Foss	REF	2012	July-Aug	84	95	6.0	7.7	0.998
25	Foss	REF	2012	Aug-Sep	97	113	9.5	15.1	1.341
26	Foss	REF	2012	Aug-Sep	96	105	8.9	11.9	1.106
27	Foss	REF	2012	Aug-Sep	82	82	5.9	5.7	0.717
28	Foss	REF	2012	Aug-Sep	90	100	7.0	10.7	1.295
29	Foss	REF	2012	Aug-Sep	89	91	7.5	8.6	0.987
30	Foss	REF	2012	Aug-Sep	77	80	4.4	5.1	0.805
31	Foss	REF	2012	Aug-Sep	80	82	4.7	5.6	0.854
32	Manke	CULT	2012	Aug-Sep	108	117	13.0	16.8	1.081
33	Manke	CULT	2012	Aug-Sep	122	127	18.6	23.2	1.203
34	Manke	CULT	2012	Aug-Sep	125	135	22.5	28.1	1.305
35	Manke	CULT	2012	Aug-Sep	120	129	18.8	26.5	1.398
36	Manke	CULT	2012	June-July	100	121	10.4	18.9	1.890
37	Manke	CULT	2012	June-July	93	112	8.9	14.1	1.526
38	Manke	CULT	2012	June-July	129	138	26.1	28.3	1.474
39	Manke	REF	2012	July-Aug	89	97	7.0	9.5	1.351
40	Manke	REF	2012	July-Aug	85	91	5.8	6.9	1.083
41	Manke	REF	2012	July-Aug	93	97	8.6	9.2	1.121
42	Manke	REF	2012	July-Aug	74	87	4.3	6.3	1.188
43	Manke	REF	2012	July-Aug	92	107	7.4	12.1	1.522
44	Manke	REF	2012	Aug-Sep	113	122	16.1	20.7	1.362
45	Manke	REF	2012	Aug-Sep	100	114	9.6	14.9	1.487
46	Manke	REF	2012	June-July	82	95	6.1	9.1	1.474
47	Manke	REF	2012	June-July	77	94	5.8	7.9	1.307
48	Manke	REF	2012	June-July	80	88	4.9	7.2	1.323
49	Manke	REF	2012	June-July	80	95	5.3	8.7	1.533
50	Manke	REF	2012	June-July	74	85	4.4	6.7	1.312
51	Manke	REF	2012	June-July	75	89	4.3	7.0	1.397
52	Manke	REF	2012	Aug-Sep	97	100	9.2	11.4	1.258
53	Manke	REF	2012	Aug-Sep	91	105	7.7	12.2	1.521
54	Manke	REF	2012	Aug-Sep	94	104	8.2	11.0	1.283

Table 2.13. Average P-values generated from sculpin recaptures and used in the Wisconsin model projections. *P-values for Rolfs were estimated by averaging comparable estimates from Foss and Manke.

Site	Plot	Year	P-value	N	Standard Error
Foss	CULT	2011	1.400	7	0.052
Foss	REF	2011	1.226	10	0.040
Foss	CULT	2012	0.904	1	NA
Foss	REF	2012	1.032	13	0.054
Manke	CULT	2012	1.411	7	0.099
Manke	REF	2012	1.345	16	0.035
Rolfs	CULT	2012	1.157*	0	NA
Rolfs	REF	2012	1.189*	0	NA

Table 2.14. List of all candidate models for 2011 P-values from sculpin recaptures. P: plot, L: staghorn sculpin length, K: number of model parameters, ΔAICc: change in AICc, W_i: Akaike weight, ΣW_i: cumulative Akaike weight, ER: evidence ratio. Shaded models: included in list of top candidate models ($\Sigma W_i \leq 0.95$).

Model	Model ID	K	AICc	ΔAICc	Wi	ΣWi	ER
P	1	3	-15.15	0	0.54	0.54	1.0
P x L	2	5	-13.62	1.53	0.25	0.79	2.2
P + L	3	4	-11.85	3.3	0.1	0.89	5.4
Null	4	2	-11.41	3.74	0.08	0.98	6.8
L	5	3	-8.78	6.38	0.02	1	27.0

Table 2.15. Model averaged parameter weights for 2011 P-values from sculpin recaptures.

Parameter	Base Model	Estimate	SE	Lower 95% CI	Upper 95% CI
Plot CULT	Plot REF	0.668	0.770	-0.840	2.177
Length	N/A	0.001	0.002	-0.003	0.006
Plot CULT x Length	Plot REF x Length	-0.005	0.004	-0.013	0.003

Table 2.16. List of all candidate models for 2012 P-values from sculpin recaptures. S: site, P: plot, L: staghorn sculpin length, K: number of model parameters, ΔAICc: change in AICc, W_i: Akaike weight, ΣW_i: cumulative Akaike weight, ER: evidence ratio. Shaded models: included in list of top candidate models ($\Sigma W_i \leq 0.95$).

Model	Model ID	K	AICc	ΔAICc	Wi	ΣWi	ER
S	1	3	-15.21	0.00	0.43	0.43	1.0
S + L	2	4	-12.94	2.27	0.14	0.57	3.1
S + P	3	4	-12.90	2.31	0.14	0.71	3.1
S + L + S x L	4	5	-11.61	3.60	0.07	0.78	6.1
S + P + S x P	5	5	-11.15	4.06	0.06	0.84	7.2
S + P + L	6	5	-10.28	4.93	0.04	0.88	10.8
S + P + L + P x L	7	6	-9.74	5.47	0.03	0.90	14.3
S + P + L + S x P + S x L	8	7	-9.34	5.87	0.02	0.93	21.5
S + P + L + S x P + P x L	9	7	-9.08	6.12	0.02	0.95	21.5
S + P + L + S x L	10	6	-8.79	6.42	0.02	0.97	21.5
S + P + L + S x P	11	6	-8.29	6.92	0.01	0.98	43.0
S + P + L + S x P + S x L + P x L	12	8	-8.27	6.94	0.01	0.99	43.0
S + P + L + S x L + P x L	13	7	-7.06	8.15	0.01	1.00	43.0
L	14	3	4.99	20.20	0.00	1.00	N/A
Null	15	2	5.20	20.40	0.00	1.00	N/A
P	16	3	5.37	20.58	0.00	1.00	N/A
P + L	17	4	7.29	22.50	0.00	1.00	N/A
P + L + P x L	18	5	9.06	24.27	0.00	1.00	N/A

Table 2.17. Model averaged parameter weights for 2012 P-values from sculpin recaptures. Bold confidence interval excludes zero and indicates an effect of site.

Parameter	Base Model	Estimate	SE	Lower 95% CI	Upper 95% CI
Site Foss	Site Manke	-0.423	0.202	**-0.818**	**-0.028**
Plot CULT	Plot REF	0.071	0.091	-0.107	0.248
Length	N/A	0.000	0.001	-0.002	0.002
Site Foss x Length	Site Manke x Length	0.001	0.001	-0.001	0.003
Site Foss x Plot CULT	Site Manke x Plot REF	-0.029	0.036	-0.100	0.041
Plot CULT x Length	Plot REF x Length	-0.001	0.001	-0.002	0.001

Table 2.18. Total biomass of prey compared to sculpin population consumption at each site and plot in 2011 and 2012. Pop. Cons.: consumption of a population of 1000 staghorn sculpin.

Month	Site	Plot	Year	Total Pop. Cons. (g)	Total Biomass for 5000 m^2 Plot (g)	Total Biomass-Total Pop. Cons.	Total Biomass/ Total Pop. Cons.	# Sculpin Supported
July	Foss	CULT	2011	22515	28441	5926	1	1263
July	Foss	REF	2011	18713	182797	164085	10	9769
Aug	Foss	CULT	2011	25649	138794	113145	5	5411
Aug	Foss	REF	2011	21068	130808	109740	6	6209
May	Foss	CULT	2012	3904	47782	43877	12	12238
May	Foss	REF	2012	5173	188248	183075	36	36389
June	Foss	CULT	2012	11254	47782	36528	4	4246
June	Foss	REF	2012	9852	188248	178396	19	19107
July	Foss	CULT	2012	14009	38670	24660	3	2760
July	Foss	REF	2012	14674	122101	107428	8	8321
Aug	Foss	CULT	2012	16020	29558	13538	2	1845
Aug	Foss	REF	2012	17293	55954	38661	3	3236
May	Manke	CULT	2012	9012	114814	105802	13	12740
May	Manke	REF	2012	7581	154380	146799	20	20364
June	Manke	CULT	2012	17182	114814	97632	7	6682
June	Manke	REF	2012	15884	154380	138496	10	9719
July	Manke	CULT	2012	22477	80958	58481	4	3602
July	Manke	REF	2012	21084	108551	87467	5	5148
Aug	Manke	CULT	2012	27480	47102	19622	2	1714
Aug	Manke	REF	2012	23262	62721	39459	3	2696
May	Rolfs	CULT	2012	7321	54731	47410	7	7476
May	Rolfs	REF	2012	5587	63629	58042	11	11388
June	Rolfs	CULT	2012	14614	54731	40117	4	3745
June	Rolfs	REF	2012	12760	63629	50869	5	4986
July	Rolfs	CULT	2012	18612	62961	44350	3	3383
July	Rolfs	REF	2012	17702	87015	69313	5	4916
Aug	Rolfs	CULT	2012	23017	71192	48174	3	3093
Aug	Rolfs	REF	2012	21958	111069	89111	5	5058

Table 2.19. Sculpin population consumption at Foss for each prey group. Biomass based on a 5000 m^2 plot. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp, Pop. Cons.: consumption of a population of 1000 staghorn sculpin.

Month	Site	Plot	Year	Category	Total (g)	AMPH (g)	BIV (g)	CORO (g)	CRAB (g)	ISO (g)	OCRUST (g)	POLY (g)	SHRI (g)
July	Foss	CULT	2011	Biomass	28441.176	6581.763	5137.969	426.961	3741.096	11.911	4496.271	6855.154	1190.051
				Pop. Cons.	22514.799	439.253	0.000	1865.257	2781.501	15900.623	0.000	1243.320	284.845
July	Foss	REF	2011	Biomass	182797.422	0.000	3355.408	154559.985	340.100	0.000	2715.869	21656.054	170.007
				Pop. Cons.	18712.864	973.881	0.000	12469.268	3495.219	0.000	0.000	1774.496	0.000
Aug	Foss	CULT	2011	Biomass	138793.783	83023.434	16318.560	4679.063	3185.937	4.675	13405.714	7368.611	10807.790
				Pop. Cons.	25648.660	15481.206	0.000	6.185	8222.403	1938.867	0.000	0.000	0.000
Aug	Foss	REF	2011	Biomass	130808.473	1253.184	5158.771	107937.468	1274.375	0.000	3033.410	11418.535	732.732
				Pop. Cons.	21068.279	1302.718	0.000	2148.863	17470.315	13.070	28.754	28.754	75.806
May	Foss	CULT	2012	Biomass	47781.682	1181.864	5639.328	2674.478	516.142	38.294	6323.654	31407.922	0.000
				Pop. Cons.	3904.305	2678.437	0.000	321.860	0.000	380.635	11.195	512.178	0.000
May	Foss	REF	2012	Biomass	188248.197	0.000	522.160	105017.830	43.012	19.147	1386.766	80945.619	313.663
				Pop. Cons.	5173.239	1815.214	0.000	2343.145	850.957	0.000	28.928	134.996	0.000
June	Foss	CULT	2012	Biomass	47781.682	1181.864	5639.328	2674.478	516.142	38.294	6323.654	31407.922	0.000
				Pop. Cons.	11253.610	7560.766	0.000	84.297	2846.635	256.132	9.727	496.054	0.000
June	Foss	REF	2012	Biomass	188248.197	0.000	522.160	105017.830	43.012	19.147	1386.766	80945.619	313.663
				Pop. Cons.	9852.360	8088.041	0.000	1144.594	0.000	0.000	0.000	619.725	0.000
July	Foss	CULT	2012	Biomass	38669.828	2000.764	7925.794	2294.320	895.258	19.147	5428.730	19922.631	183.183
				Pop. Cons.	14009.359	273.254	0.000	1162.721	1717.350	9911.285	0.000	767.649	177.100
July	Foss	REF	2012	Biomass	122101.333	1409.832	1682.374	70108.571	21.506	9.574	1981.767	46547.695	340.015
				Pop. Cons.	14673.807	763.675	0.000	9777.853	2740.797	0.000	0.000	1391.482	0.000
Aug	Foss	CULT	2012	Biomass	29557.973	2819.664	10212.260	1914.162	1274.375	0.000	4533.806	8437.341	366.366
				Pop. Cons.	16019.881	10146.329	0.000	3.573	4749.932	1120.048	0.000	0.000	0.000
Aug	Foss	REF	2012	Biomass	55954.468	2819.664	2842.588	35199.312	0.000	0.000	2576.767	12149.771	366.366
				Pop. Cons.	17293.038	1195.596	0.000	1986.509	13967.419	12.814	28.190	28.190	74.320

Table 2.20. Sculpin population consumption at Manke for each prey group. Biomass based on a 5000 m^2 plot. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp, Pop. Cons.: consumption of a population of 1000 staghorn sculpin.

Month	Site	Plot	Year	Category	Total (g)	AMPH (g)	BIV (g)	CORO (g)	CRAB (g)	ISO (g)	OCRUST (g)	POLY (g)	SHRI (g)
May	Manke	CULT	2012	Biomass	114813.600	517.066	8250.127	10163.016	236.565	76.588	9762.835	80945.619	4861.784
				Pop. Cons.	9011.900	4329.223	77.574	1989.281	357.125	2019.407	34.619	93.889	110.781
May	Manke	REF	2012	Biomass	154380.018	960.265	3655.120	66148.752	86.024	0.000	5491.594	77881.431	156.832
				Pop. Cons.	7580.865	2023.531	0.000	468.779	243.799	167.504	158.184	4514.791	4.275
June	Manke	CULT	2012	Biomass	114813.600	517.066	8250.127	10163.016	236.565	76.588	9762.835	80945.619	4861.784
				Pop. Cons.	17181.909	4064.456	34.155	1056.910	8639.341	1115.733	0.000	2271.314	0.000
June	Manke	REF	2012	Biomass	154380.018	960.265	3655.120	66148.752	86.024	0.000	5491.594	77881.431	156.832
				Pop. Cons.	15884.411	846.651	0.000	1522.418	6602.322	4536.184	0.000	2376.836	0.000
July	Manke	CULT	2012	Biomass	80957.749	415.181	14495.245	5453.706	755.470	38.294	6724.295	48263.287	4812.269
				Pop. Cons.	22476.542	3941.566	0.000	345.440	5608.008	171.633	0.000	392.825	12017.071
July	Manke	REF	2012	Biomass	108550.605	2516.556	3985.821	45994.969	520.902	0.000	5583.503	48221.791	1727.062
				Pop. Cons.	21083.940	1745.996	0.000	10163.166	6003.631	3118.916	0.000	52.231	0.000
Aug	Manke	CULT	2012	Biomass	47101.897	313.296	20740.363	744.396	1274.375	0.000	3685.756	15580.956	4762.755
				Pop. Cons.	27480.182	1117.099	0.000	113.545	25767.041	78.719	0.000	0.000	403.779
Aug	Manke	REF	2012	Biomass	62721.191	4072.848	4316.522	25841.187	955.781	0.000	5675.412	18562.150	3297.292
				Pop. Cons.	23262.115	11893.629	0.000	420.690	10499.084	400.865	36.683	0.000	11.164

Table 2.21. Sculpin population consumption at Rolfs for each prey group. Biomass based on a 5000 m^2 plot. AMPH: non-corophium amphipods, BIV: bivalves, CORO: corophium amphipods, CRAB: crabs, ISO: isopods, OCRUST: other crustaceans, POLY: polychaetes, SHRI: shrimp, Pop. Cons.: consumption of a population of 1000 staghorn sculpin.

Month	Site	Plot	Year	Category	Total (g)	AMPH (g)	BIV (g)	CORO (g)	CRAB (g)	ISO (g)	OCRUST (g)	POLY (g)	SHRI (g)
May	Rolfs	CULT	2012	Biomass	54731.078	73.867	3446.256	0.000	193.553	842.471	443.765	28088.385	21642.781
				Pop. Cons.	7321.188	4048.131	51.360	0.000	0.000	1447.429	0.000	0.000	1774.267
May	Rolfs	REF	2012	Biomass	63628.970	221.600	10652.063	1604.687	924.755	114.882	15226.694	34727.458	156.832
				Pop. Cons.	5587.338	1247.271	0.000	811.274	0.000	2759.824	27.374	741.594	0.000
June	Rolfs	CULT	2012	Biomass	54731.078	73.867	3446.256	0.000	193.553	842.471	443.765	28088.385	21642.781
				Pop. Cons.	14613.723	4060.121	88.116	115.229	142.341	1931.777	393.134	2331.689	5551.317
June	Rolfs	REF	2012	Biomass	63628.970	221.600	10652.063	1604.687	924.755	114.882	15226.694	34727.458	156.832
				Pop. Cons.	12760.459	1600.802	0.000	126.379	2952.675	7766.570	279.566	0.000	34.467
July	Rolfs	CULT	2012	Biomass	62961.405	506.877	10987.859	0.000	733.964	423.573	776.377	30834.501	18698.254
				Pop. Cons.	18611.638	430.824	180.997	94.322	13449.603	790.040	1655.855	1427.583	582.413
July	Rolfs	REF	2012	Biomass	87015.414	3073.102	29813.848	7018.594	1004.169	214.124	9390.566	23854.591	12646.419
				Pop. Cons.	17702.107	3053.160	15.551	2736.958	217.713	6546.929	3924.010	1073.012	134.774
Aug	Rolfs	CULT	2012	Biomass	71191.732	939.888	18529.462	0.000	1274.375	4.675	1108.989	33580.616	15753.727
				Pop. Cons.	23017.471	1369.641	0.000	219.192	10791.472	0.000	215.492	0.000	10421.674
Aug	Rolfs	REF	2012	Biomass	111069.422	5639.328	48639.837	14037.188	1274.375	4.675	18004.755	16874.682	6594.584
				Pop. Cons.	21958.049	881.957	24.257	5243.289	9354.377	390.696	451.345	6.931	5605.196

Figure 2.1. Nets and tubes at a geoduck aquaculture site in South Puget Sound, Washington.

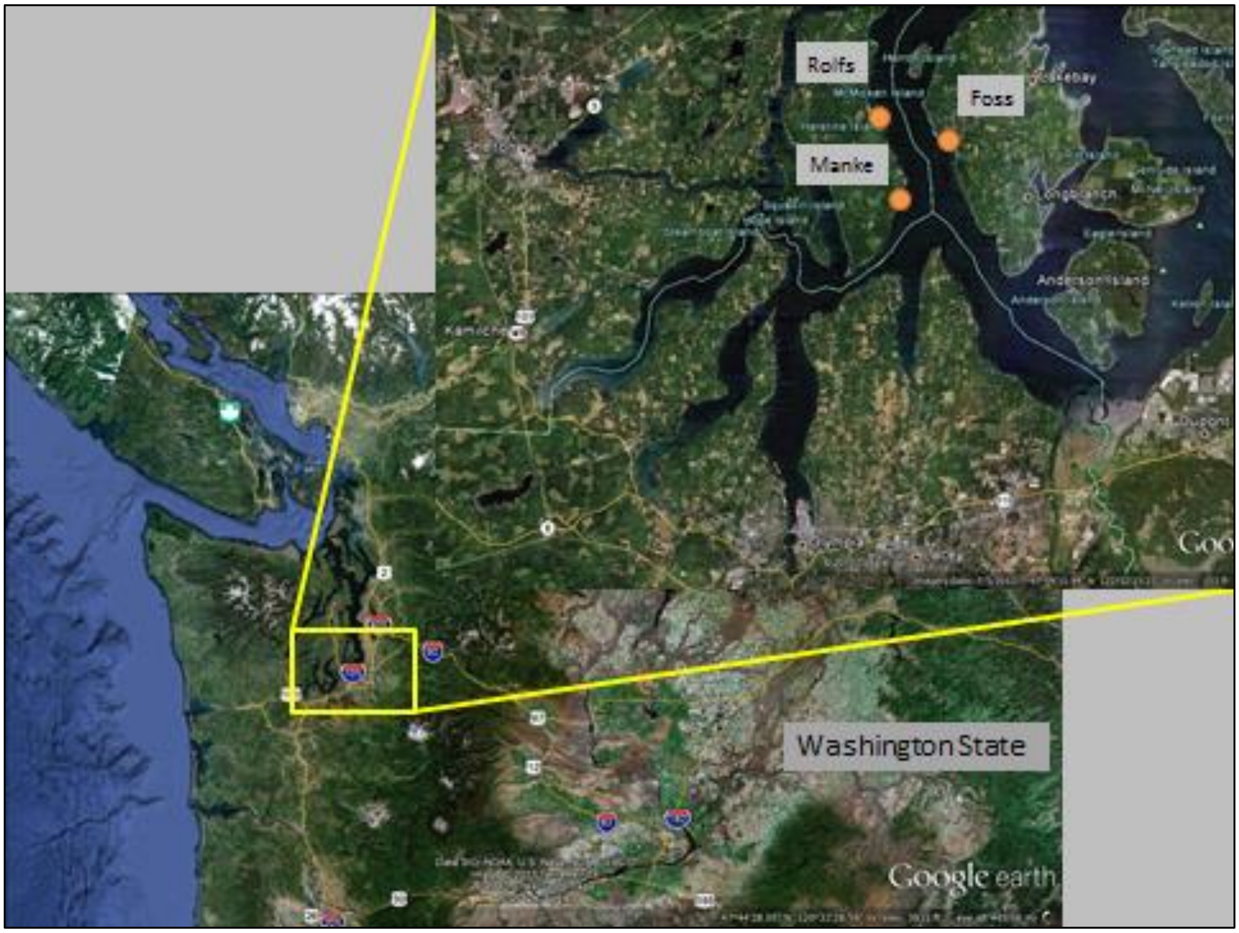

Figure 2.2. Location of research sites: Foss, Manke and Rolfs.

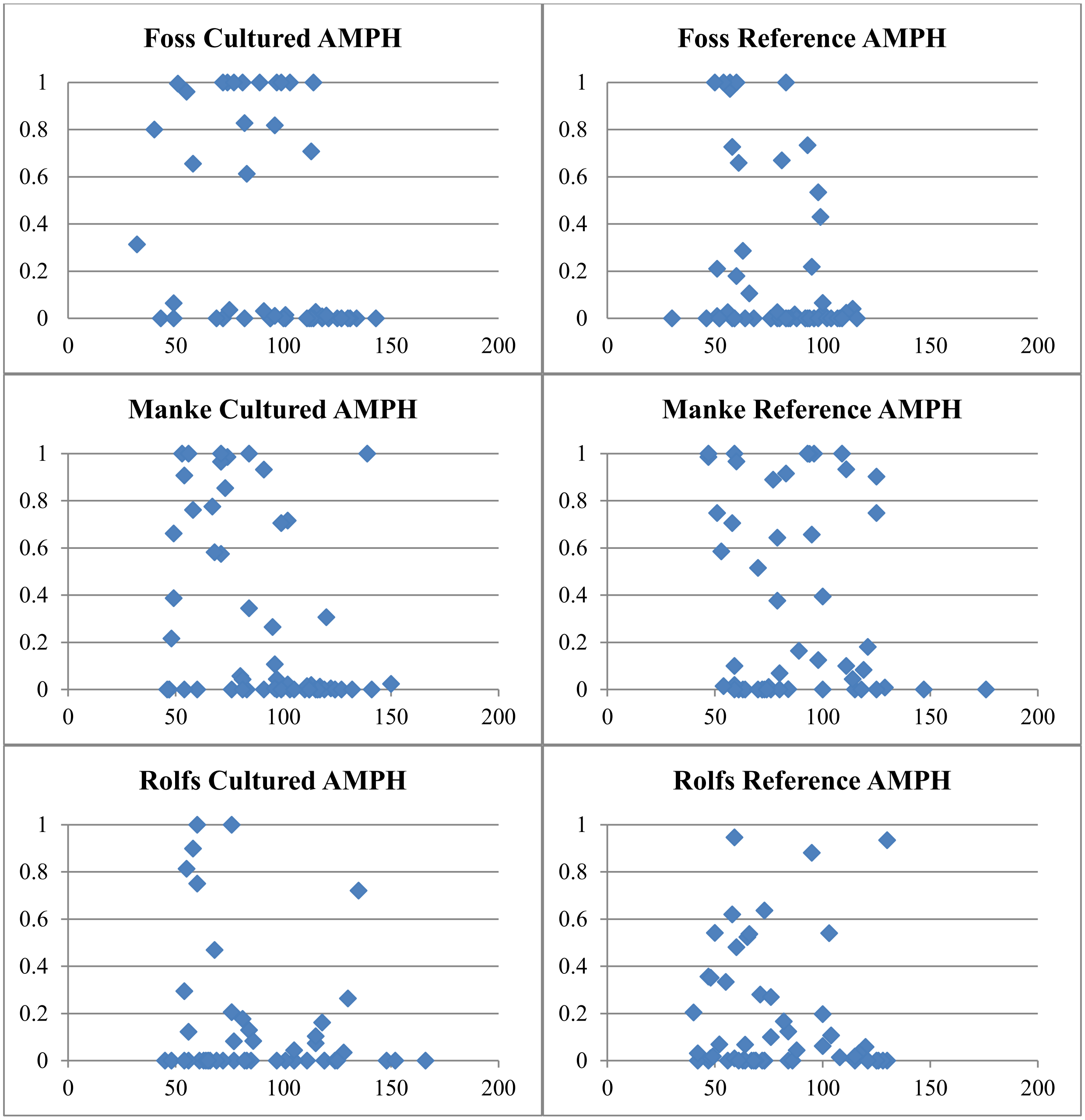

Figure 2.3a. Scatter plots of individual staghorn sculpin length (mm, horizontal axes) compared to the relative mass contribution (proportion of total prey mass in individual sculpin stomachs; vertical axes) of the amphipod (AMPH) prey group at Foss, Manke and Rolfs, cultured and reference plots.

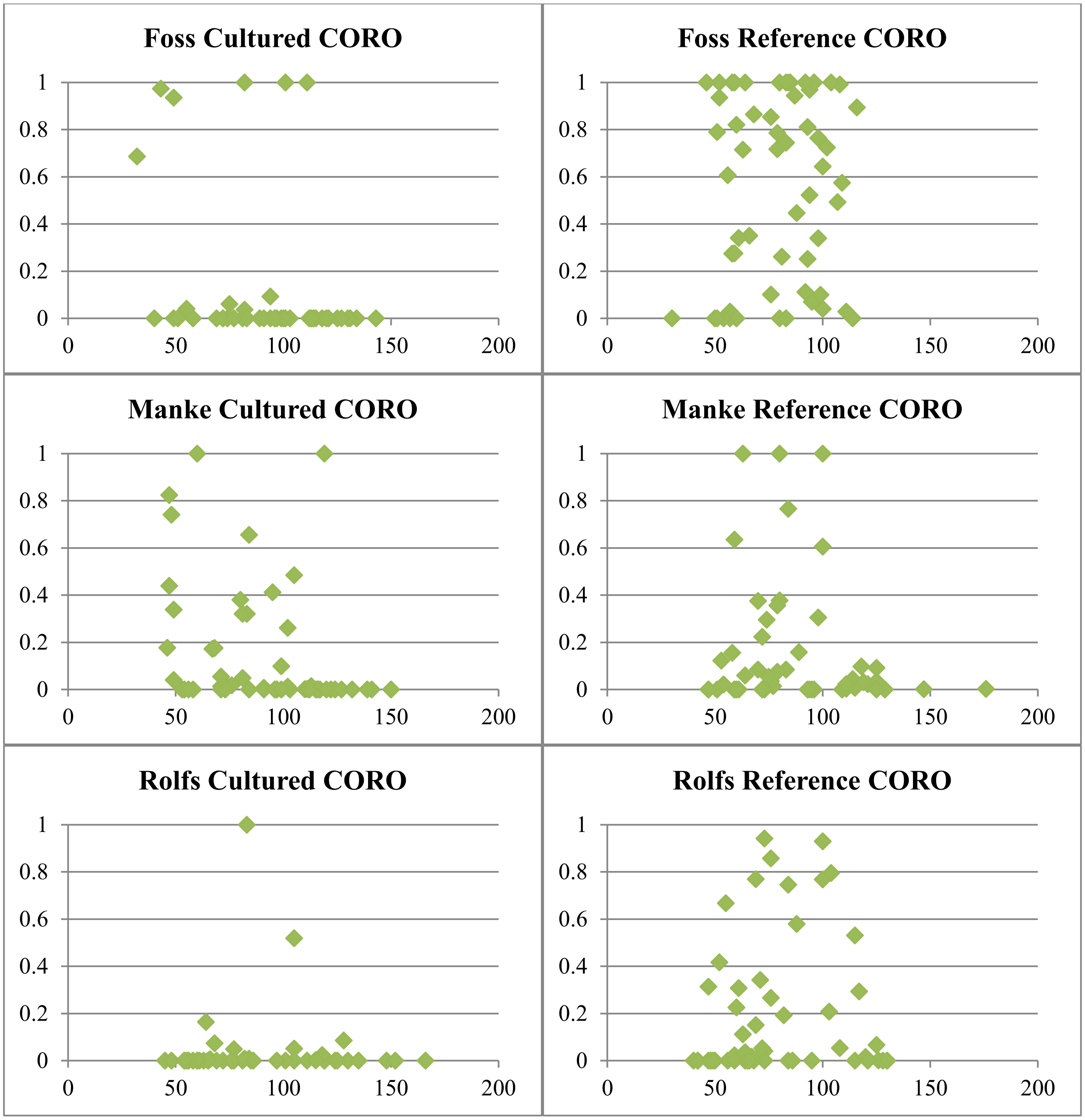

Figure 2.3b. Scatter plots of individual staghorn sculpin length (mm, horizontal axes) compared to the relative mass contribution (proportion of total prey mass in individual sculpin stomachs; vertical axes) of the corophium amphipod (CORO) prey group at Foss, Manke and Rolfs, cultured and reference plots.

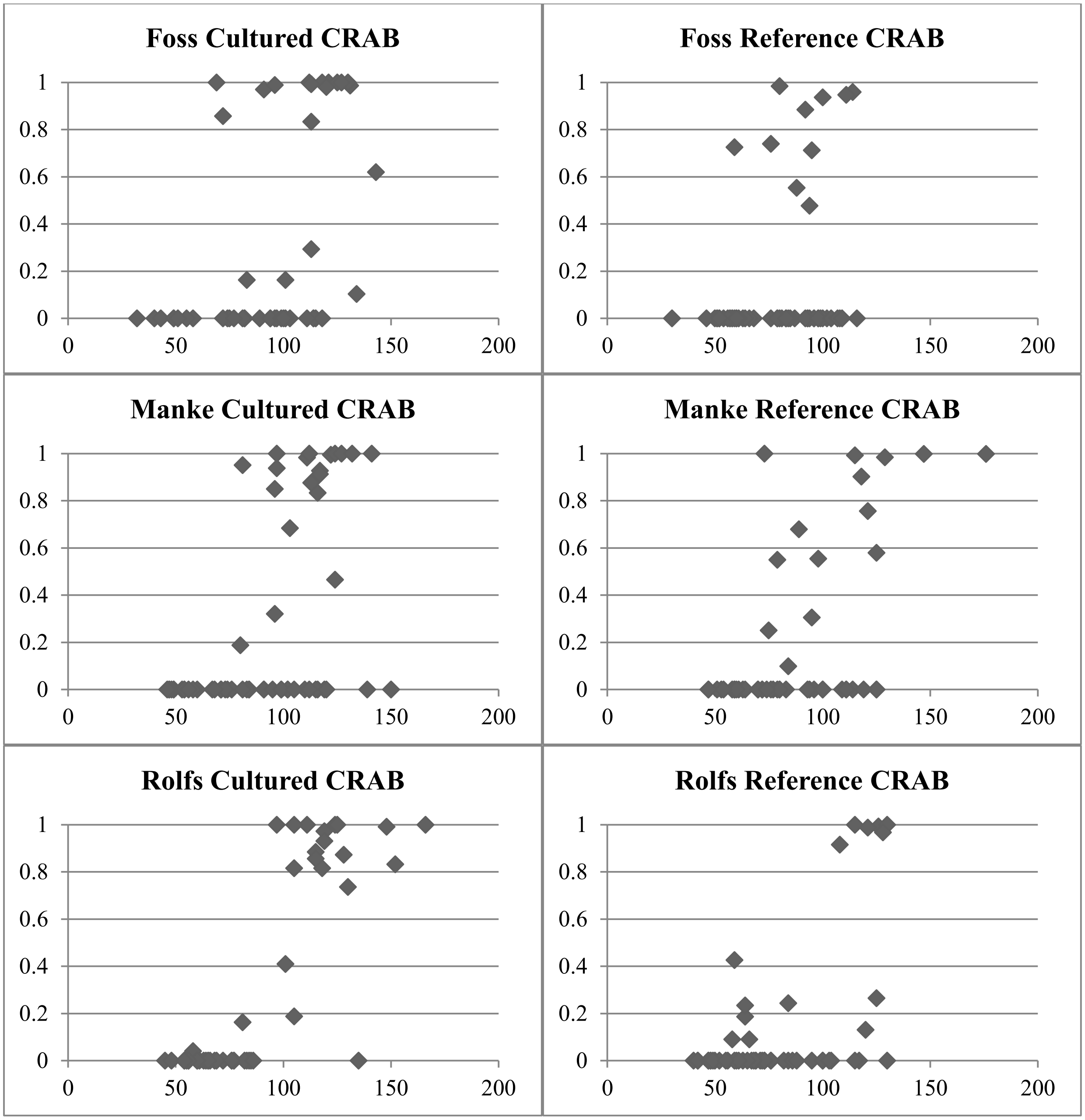

Figure 2.3c. Scatter plots of individual staghorn sculpin length (mm, horizontal axes) compared to the relative mass contribution (proportion of total prey mass in individual sculpin stomachs; vertical axes) of the crab prey group at Foss, Manke and Rolfs, cultured and reference plots.

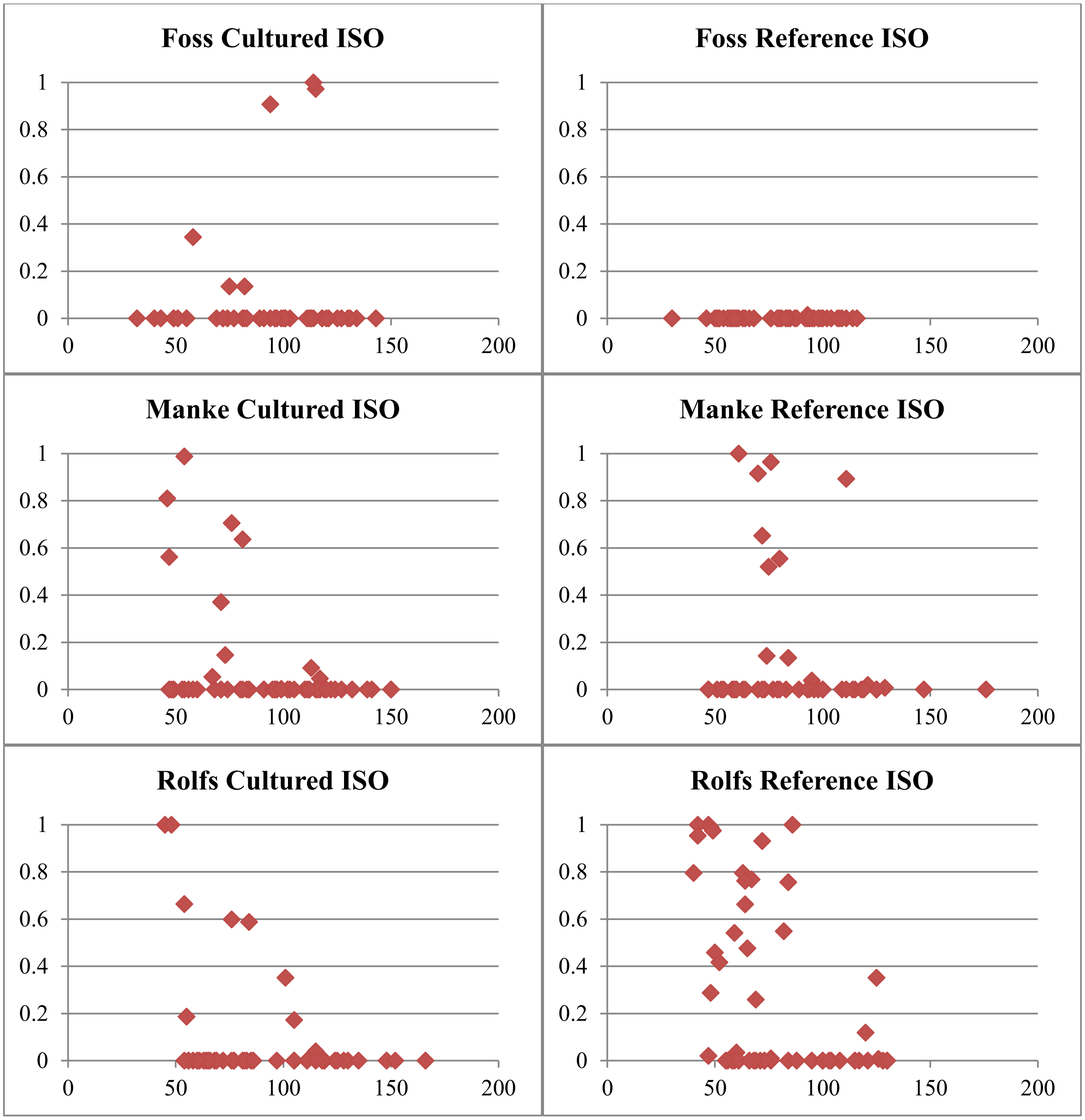

Figure 2.3d. Scatter plots of individual staghorn sculpin length (mm, horizontal axes) compared to the relative mass contribution (proportion of total prey mass in individual sculpin stomachs; vertical axes) of the isopod (ISO) prey group at Foss, Manke and Rolfs, cultured and reference plots.

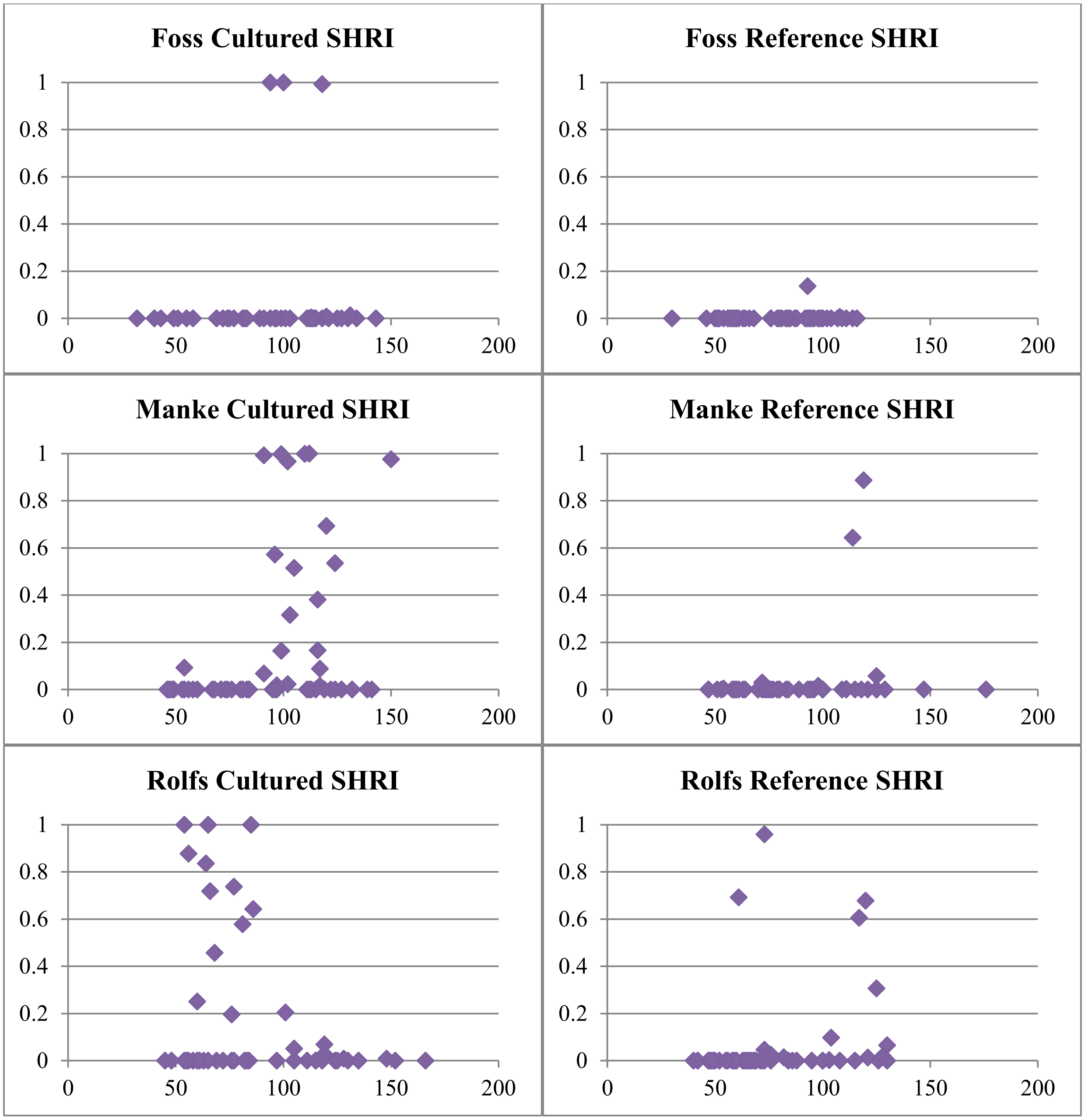

Figure 2.3e. Scatter plots of individual staghorn sculpin length (mm, horizontal axes) compared to the relative mass contribution (proportion of total prey mass in individual sculpin stomachs; vertical axes) of the shrimp (SHRI) prey group at Foss, Manke and Rolfs, cultured and reference plots.

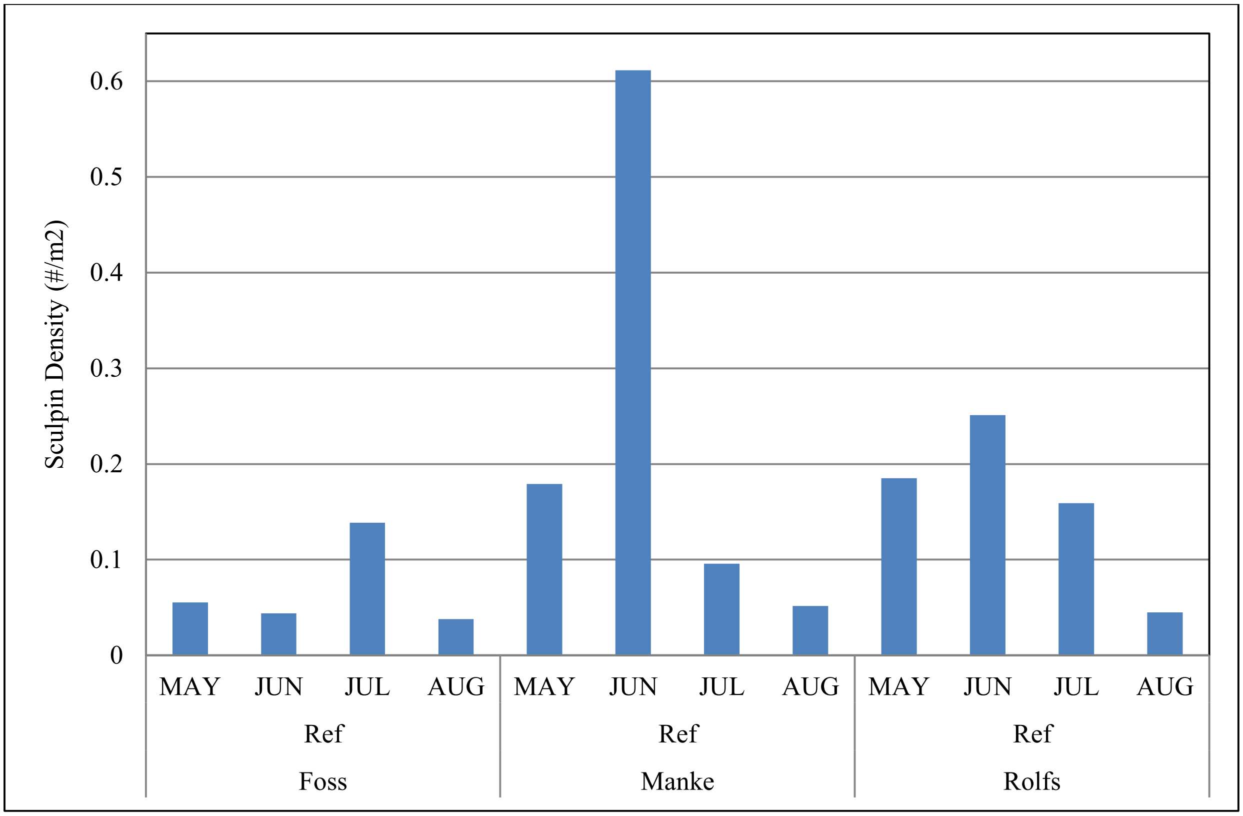

Figure 2.4. Average densities of staghorn sculpin from seining events (n=136) at reference plots in May-August 2012.

Pump handle

Outflow point with removable sampling bag

Pump base with 150 micron mesh covering the water intake area

Sampled area contained within the opening under the pump base

Figure 2.5. Pumping device for sampling epibenthos.

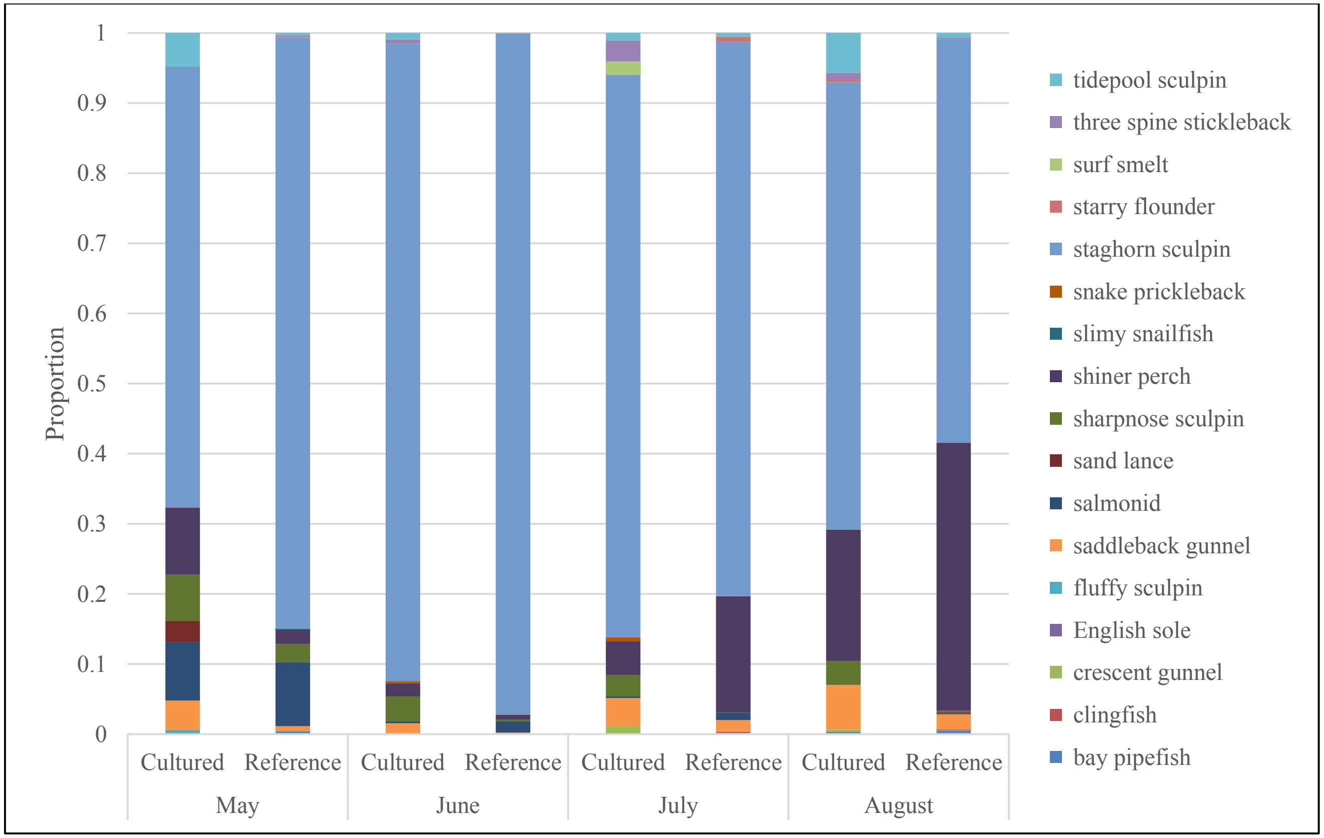

Figure 2.6. Relative abundances of all fish captured in beach seines at cultured and reference plots in 2012.

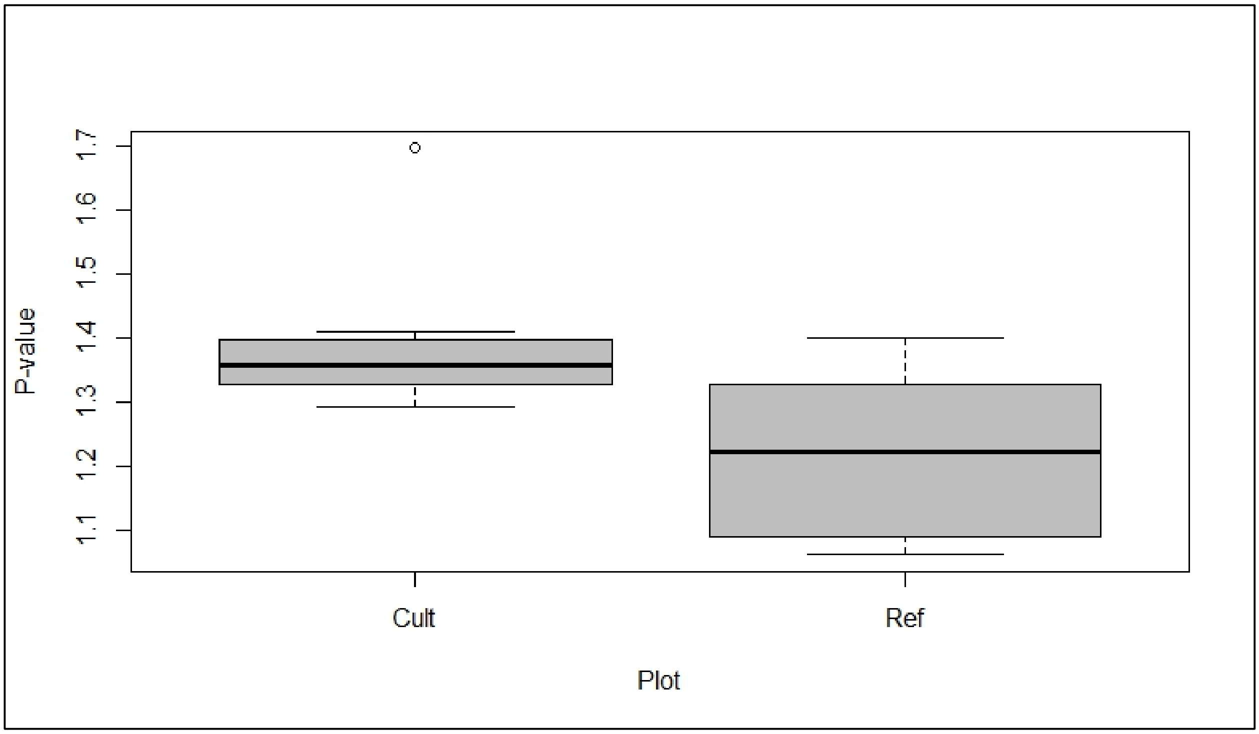

Figure 2.7. P-values (indices of feeding rate) generated by the Wisconsin bioenergetics model for sculpin recaptures at Foss in 2011.

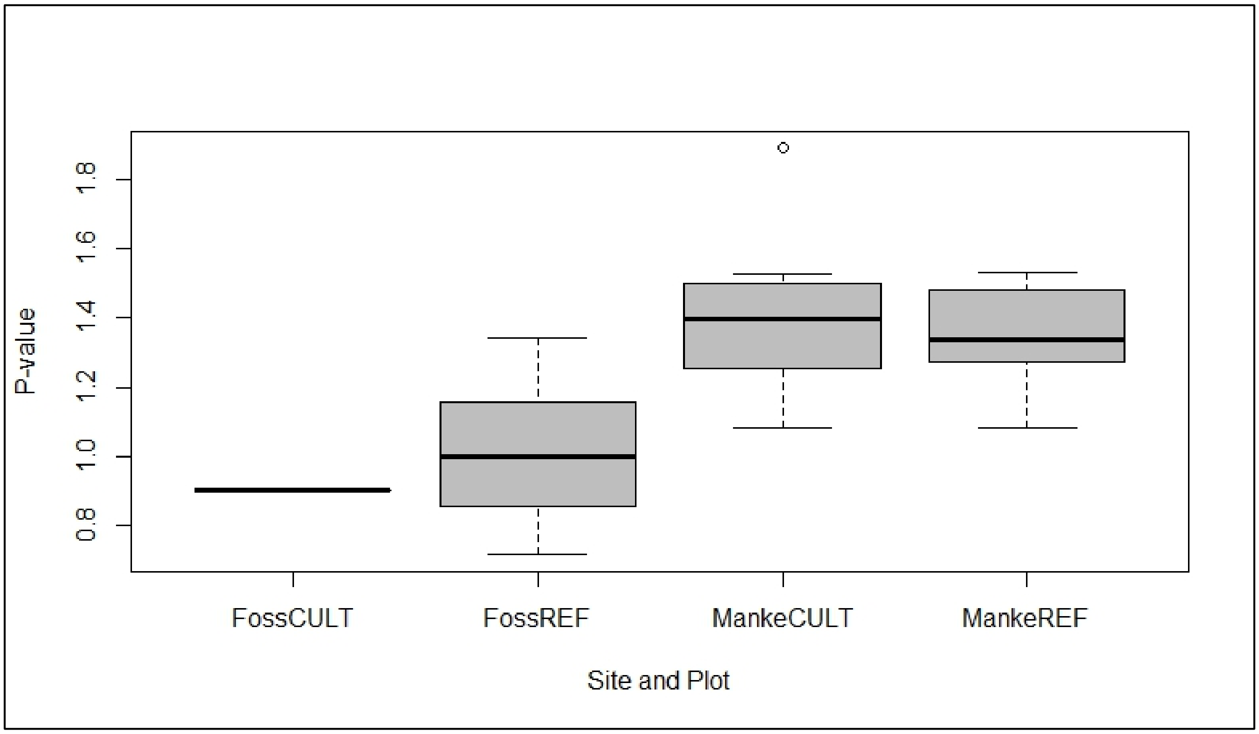

Figure 2.8. P-values (indices of feeding rate) generated by the Wisconsin bioenergetics model for sculpin recaptures at Foss and Manke in 2012.

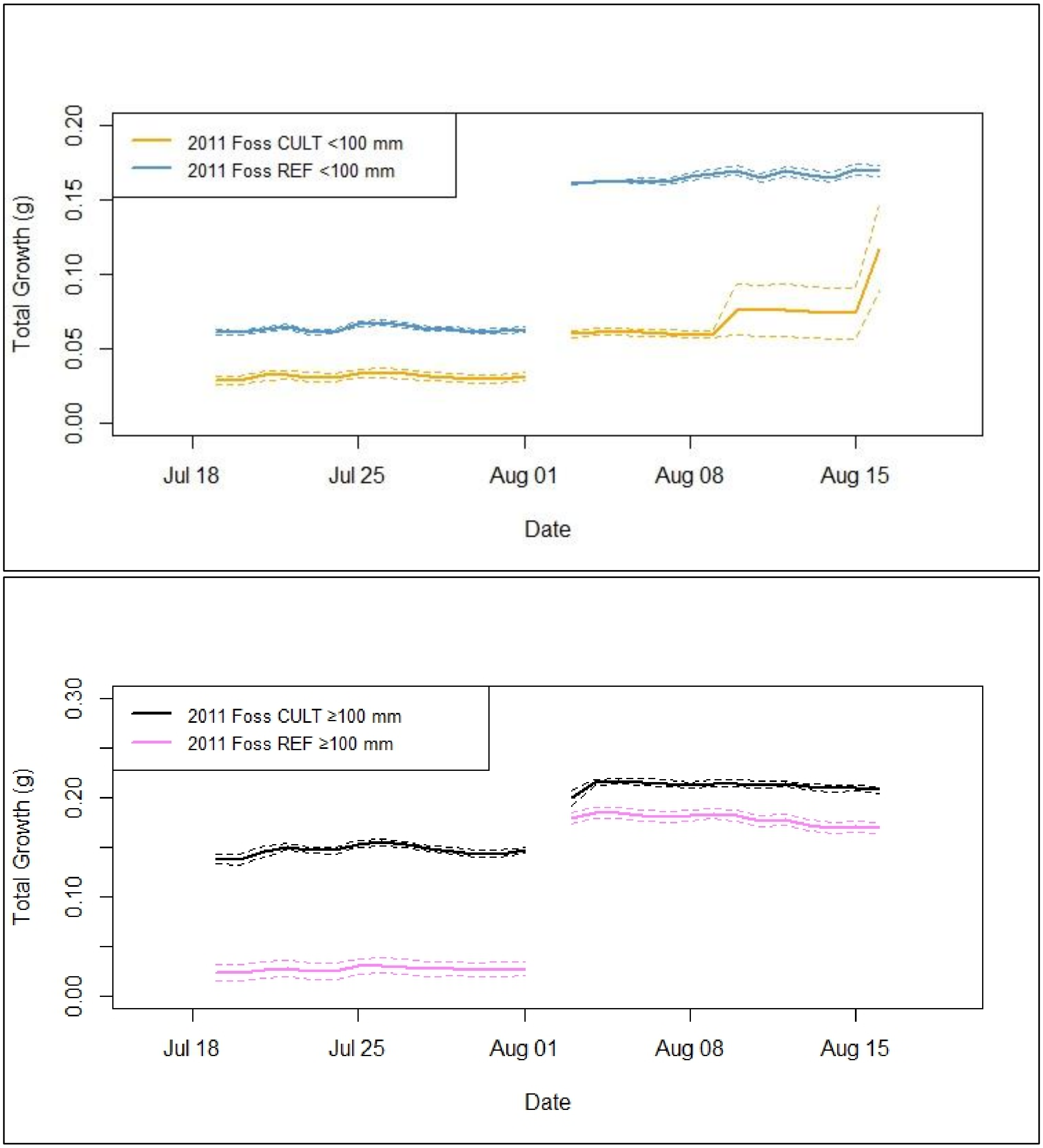

Figure 2.9. Wisconsin model projections for individual total growth of the two modeled size classes of sculpin at Foss cultured and reference plots in 2011. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

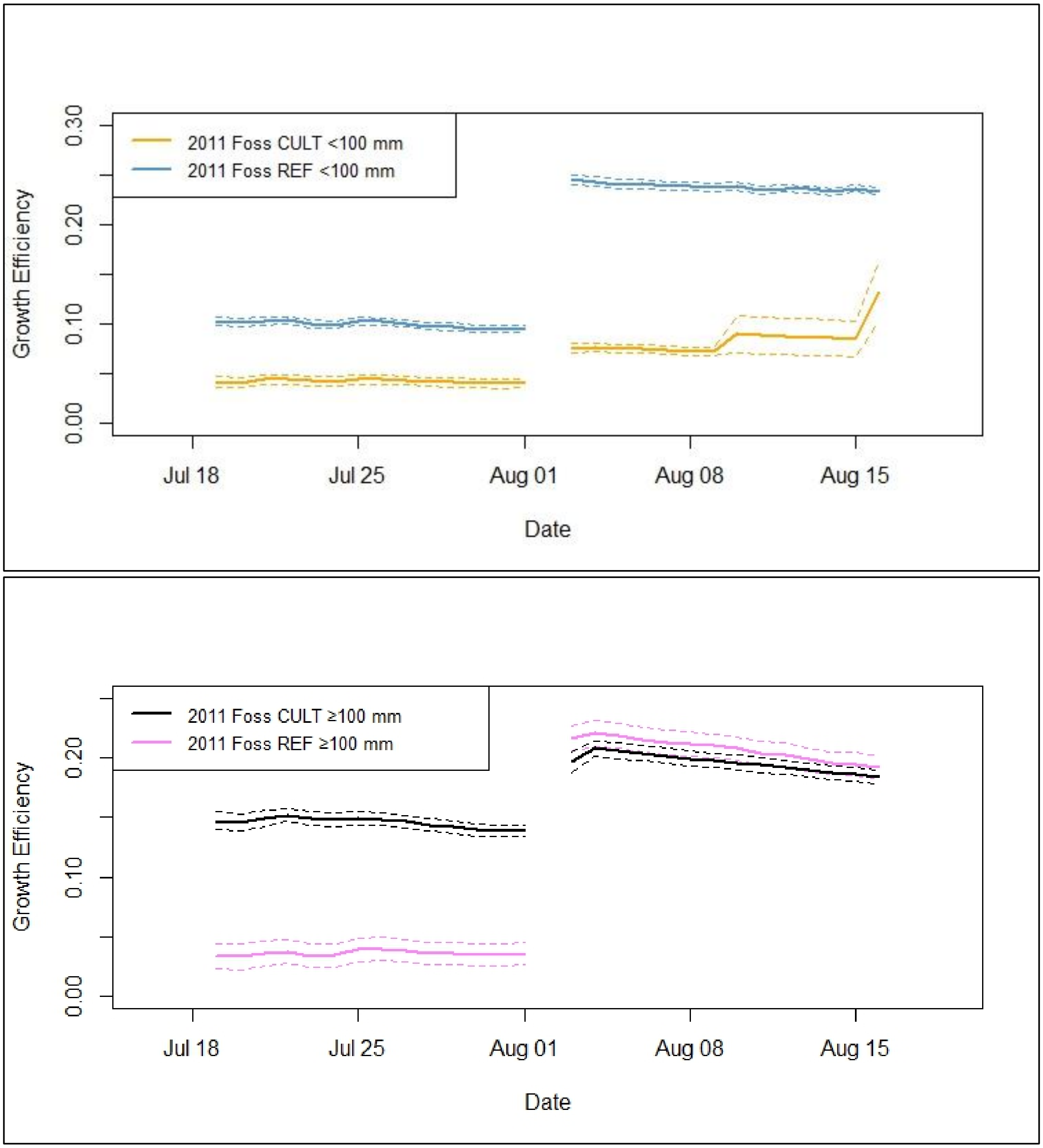

Figure 2.10. Wisconsin model projections for growth efficiency of the two modeled size classes of sculpin at Foss cultured and reference plots in 2011. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

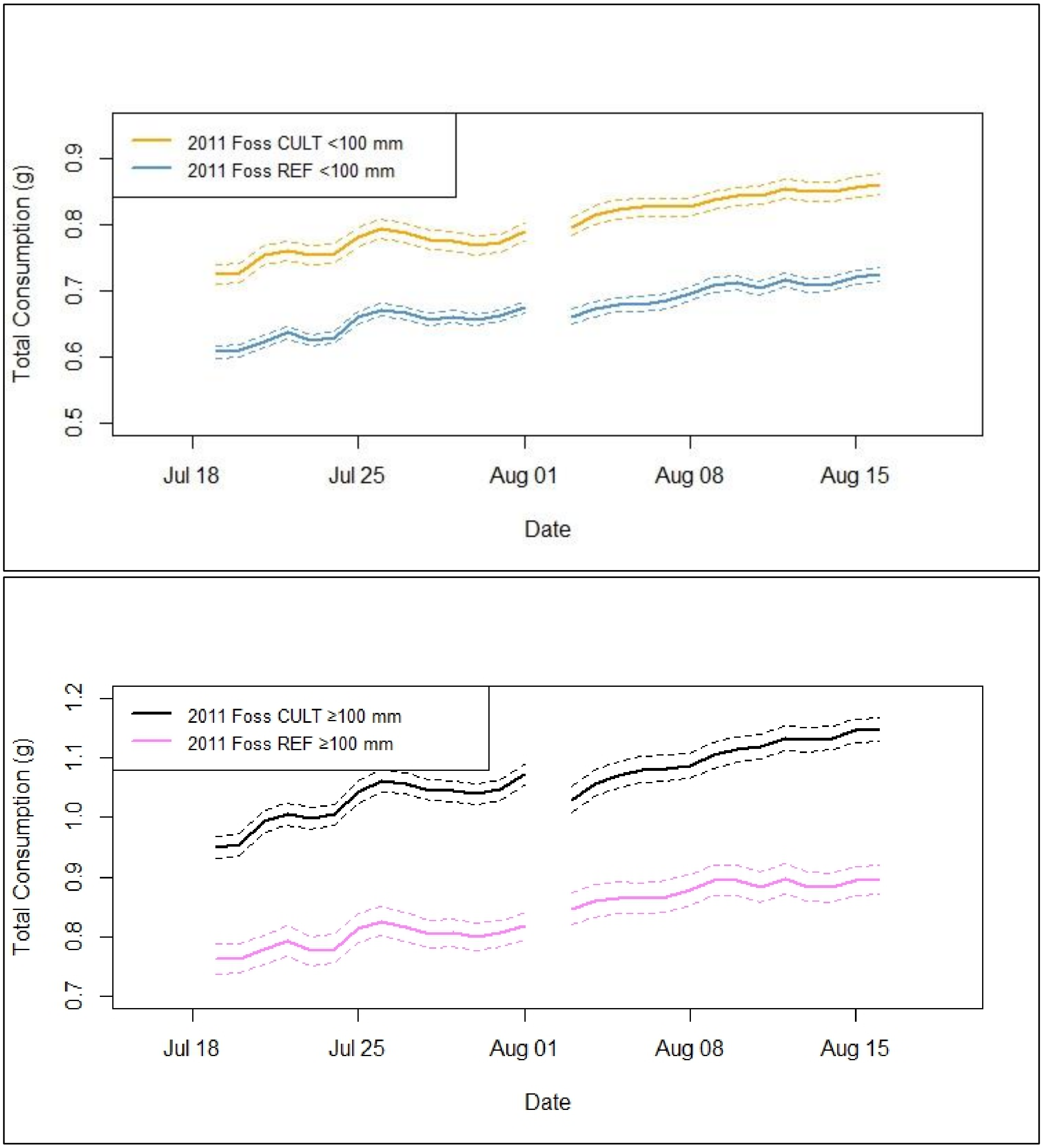

Figure 2.11. Wisconsin model projections for individual total consumption of the two modeled size classes of sculpin at Foss cultured and reference plots in 2011. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

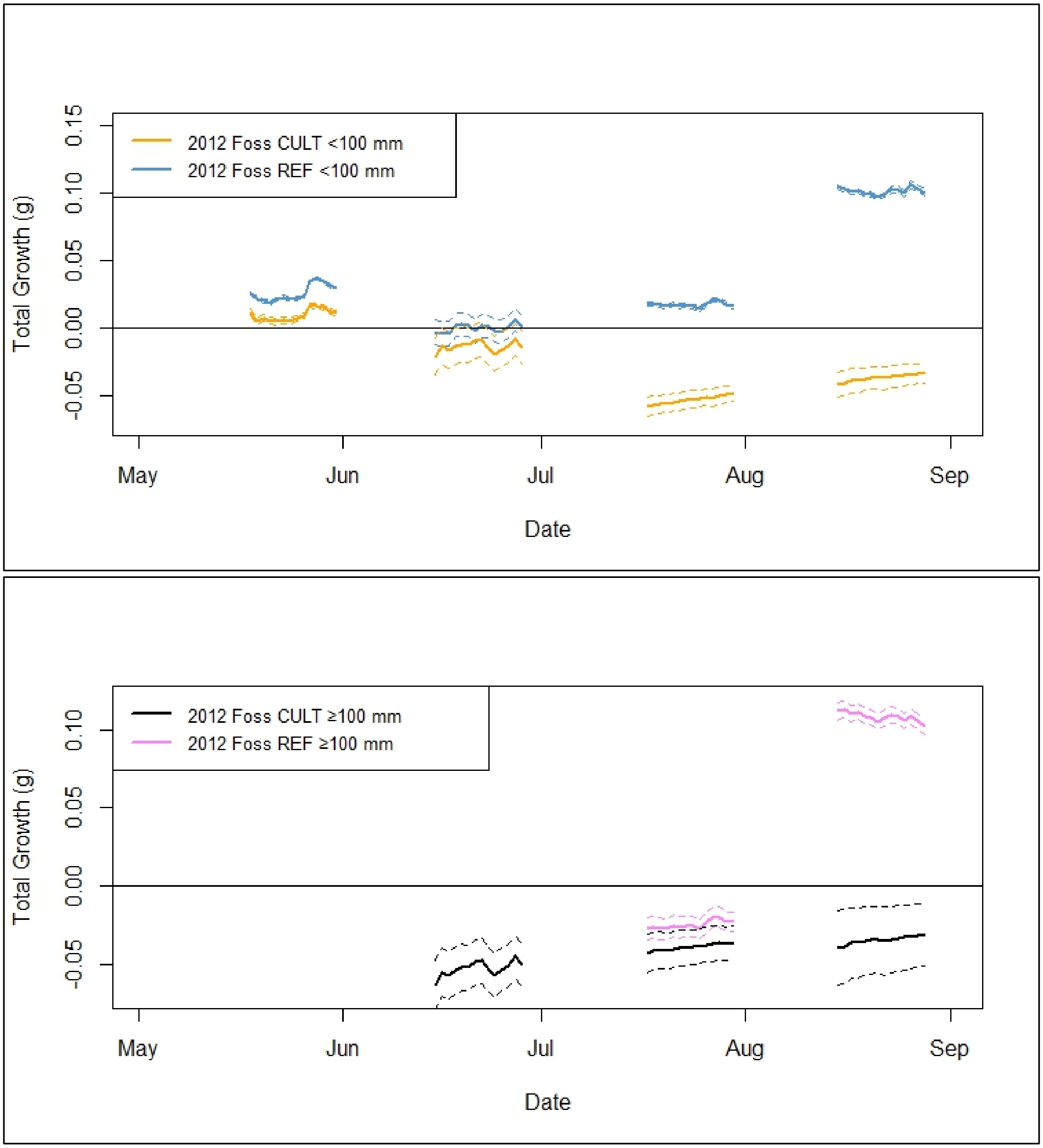

Figure 2.12. Wisconsin model projections for individual total growth of the two modeled size classes of sculpin at Foss cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

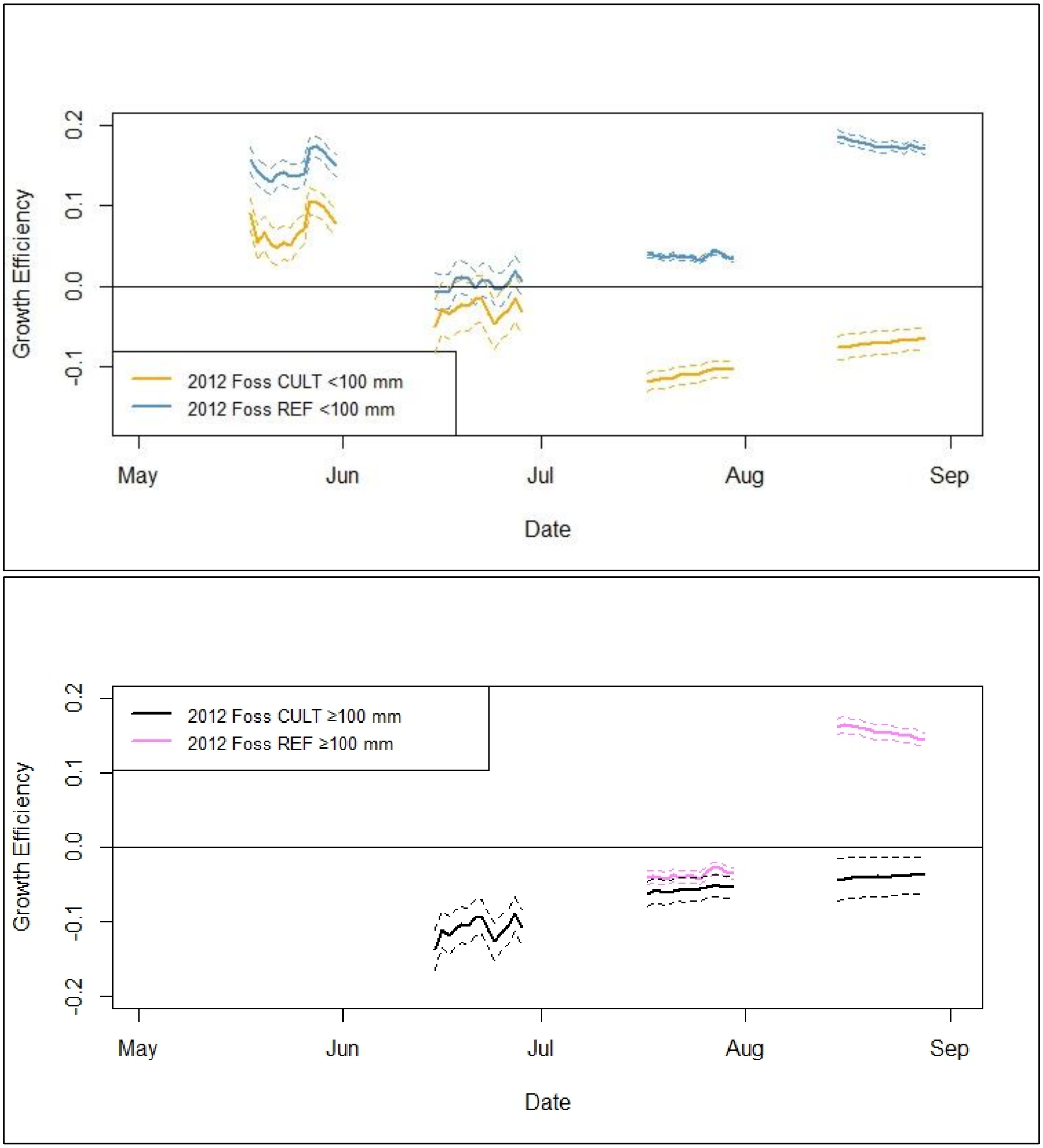

Figure 2.13. Wisconsin model projections for growth efficiency of the two modeled size classes of sculpin at Foss cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

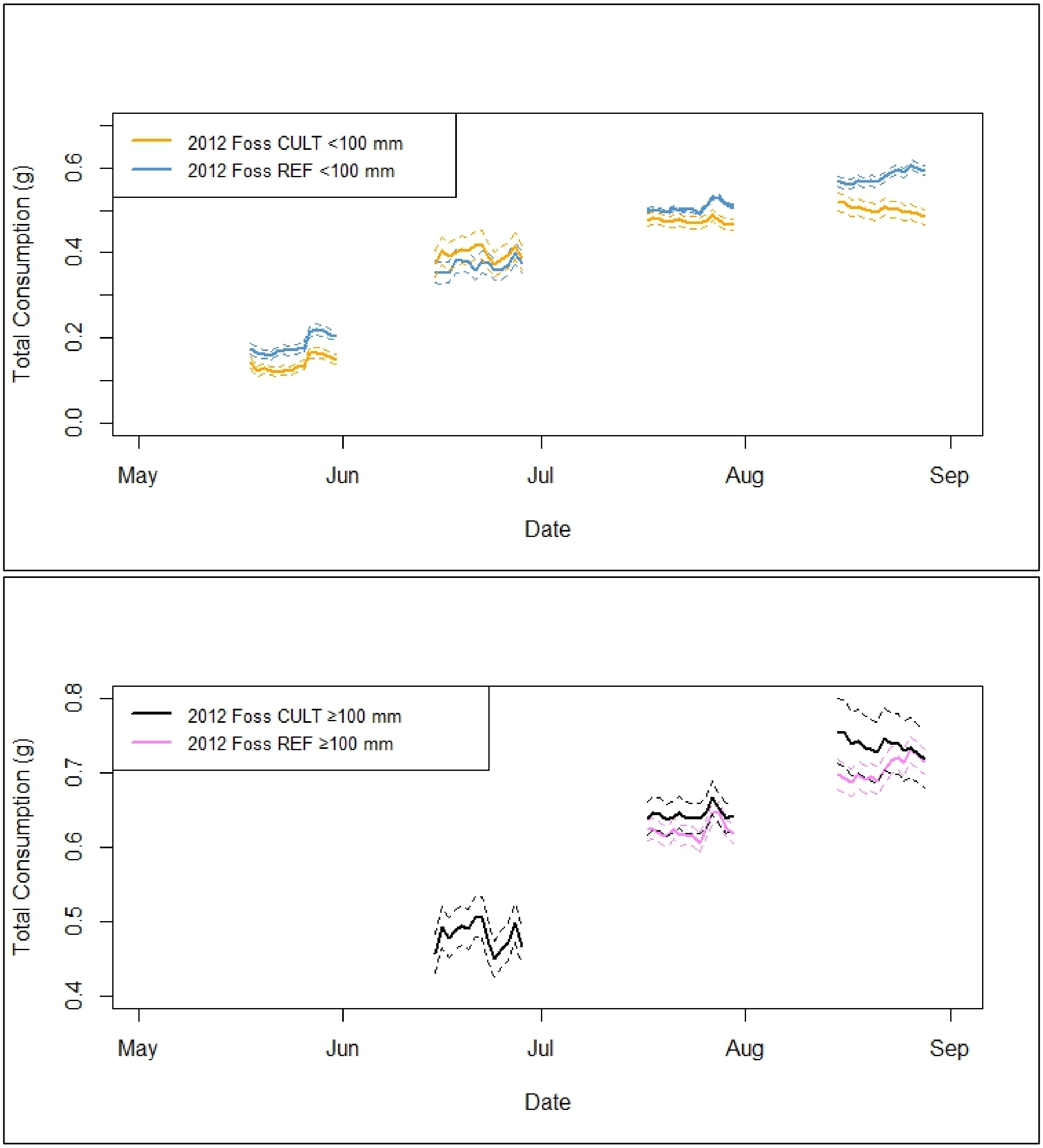

Figure 2.14. Wisconsin model projections for individual total consumption of the two modeled size classes of sculpin at Foss cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

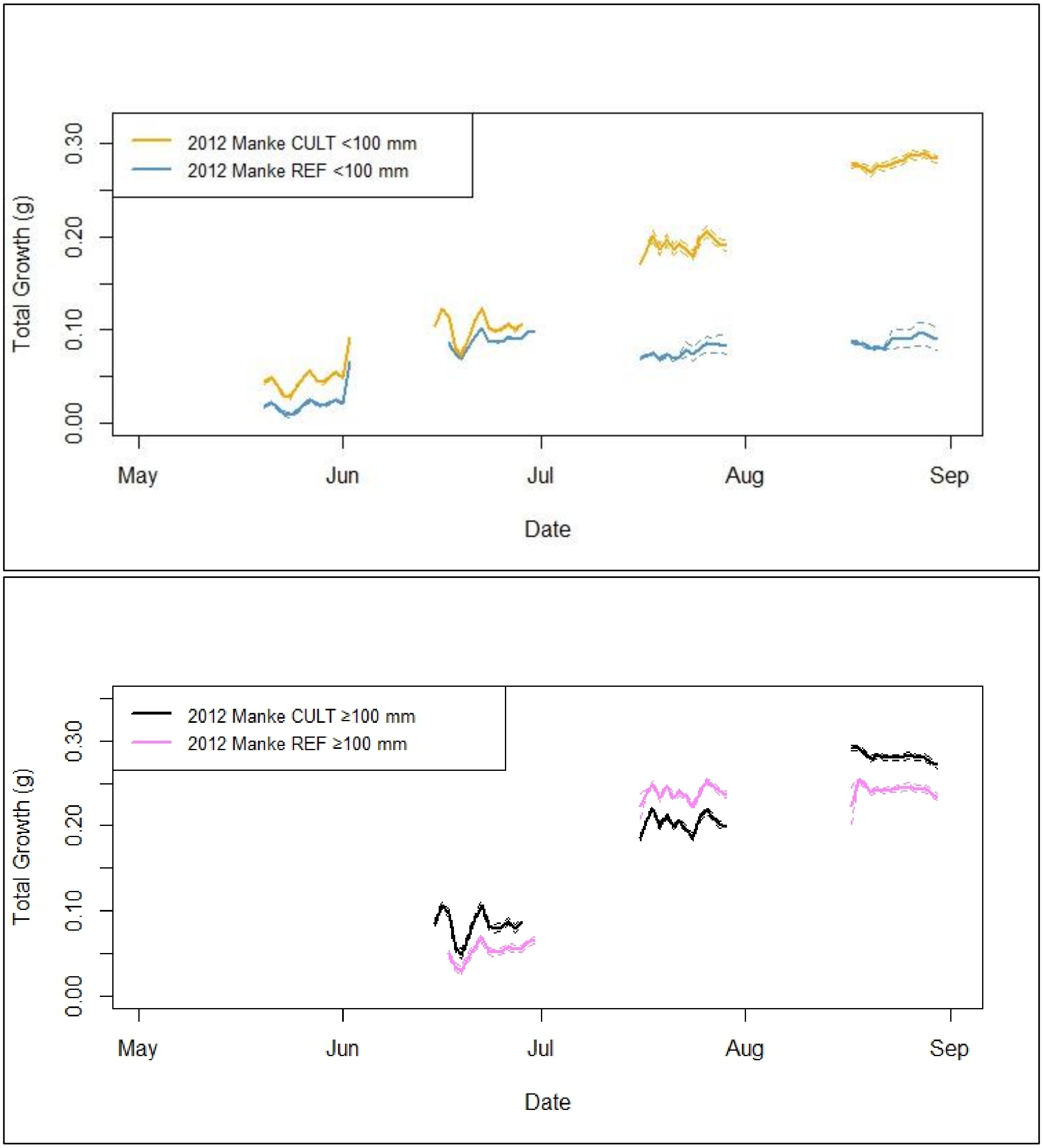

Figure 2.15. Wisconsin model projections for individual total growth of the two modeled size classes of sculpin at Manke cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

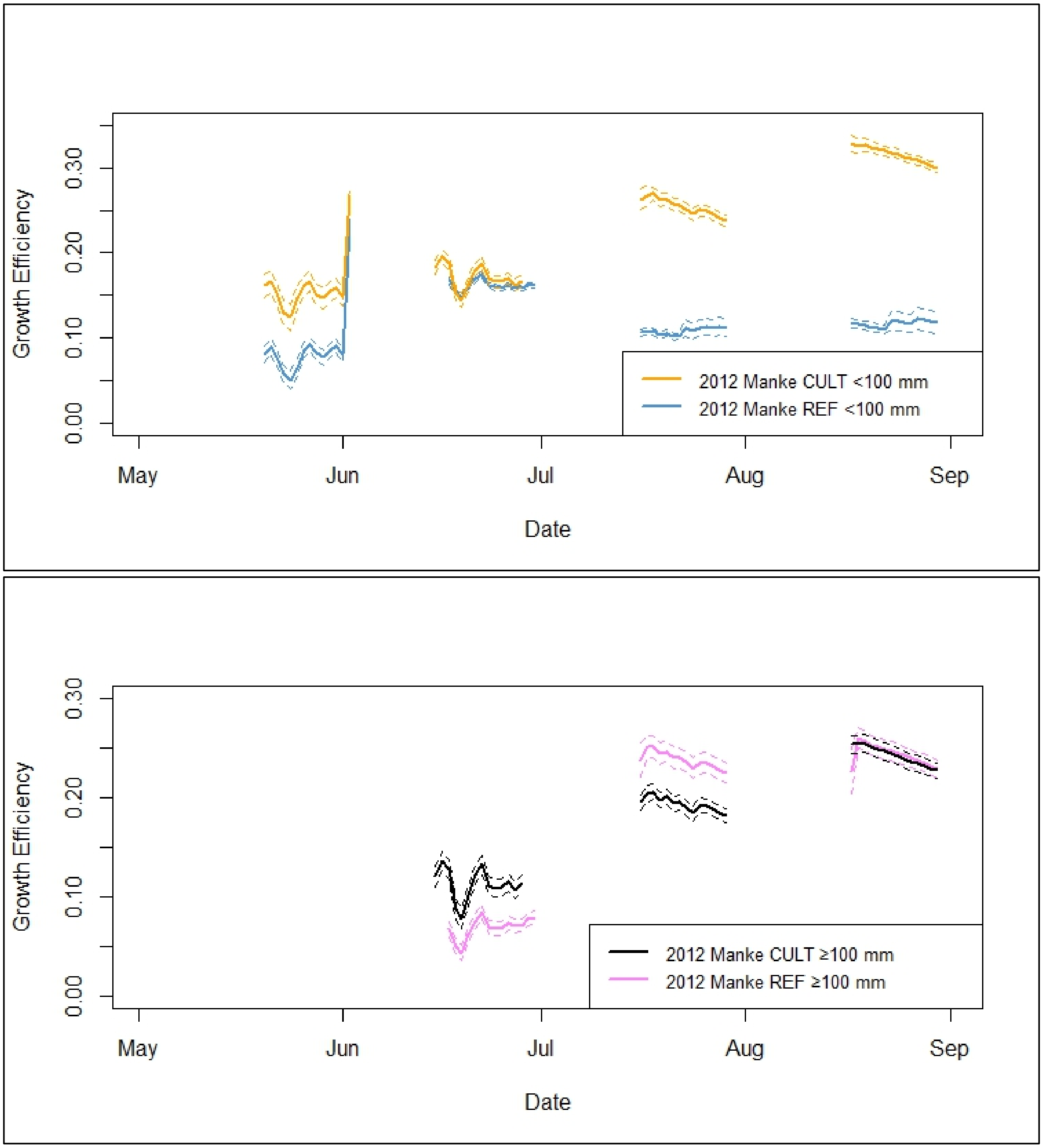

Figure 2.16. Wisconsin model projections for growth efficiency of the two modeled size classes of sculpin at Manke cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

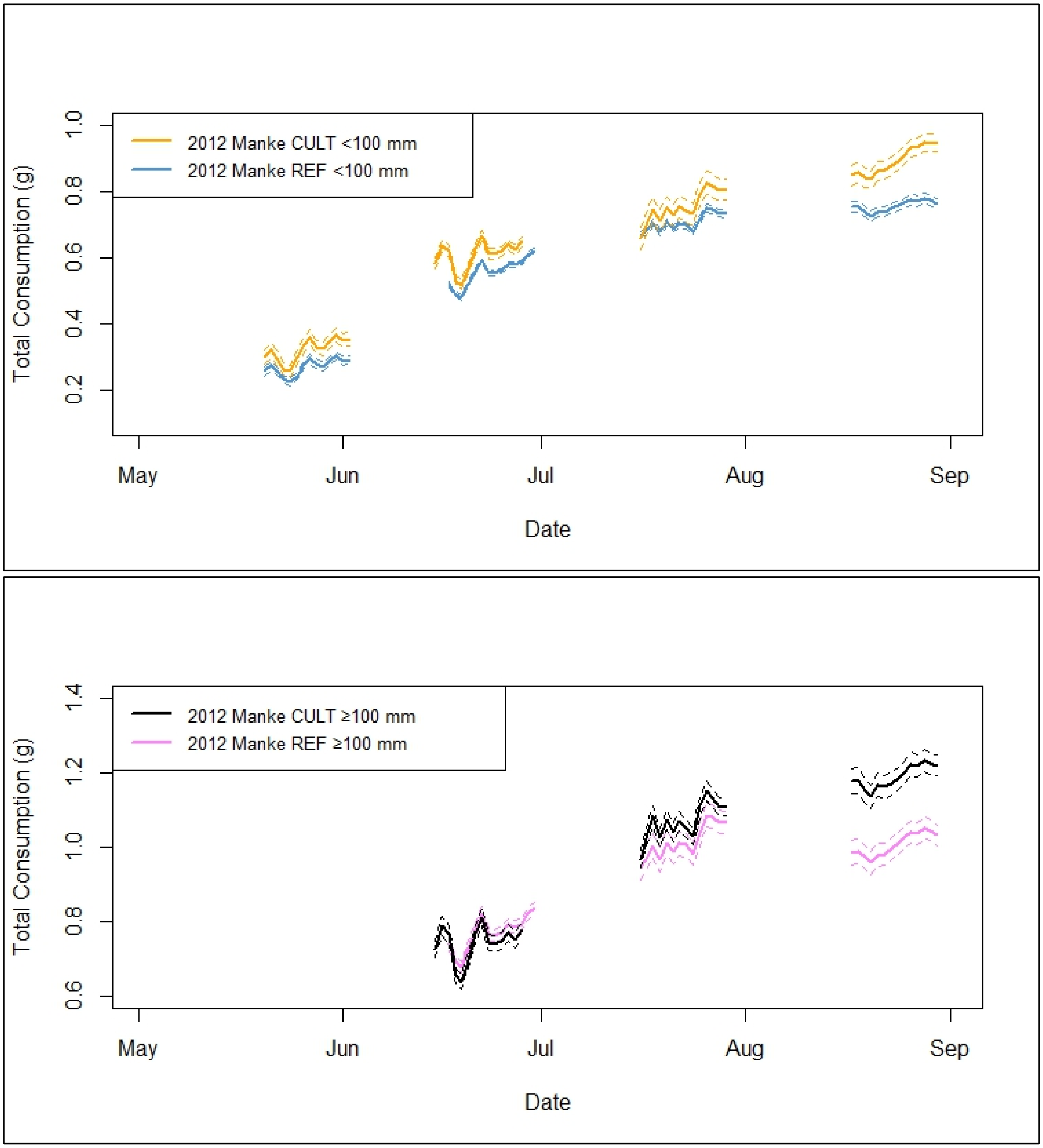

Figure 2.17. Wisconsin model projections for individual total consumption of the two modeled size classes of sculpin at Manke cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

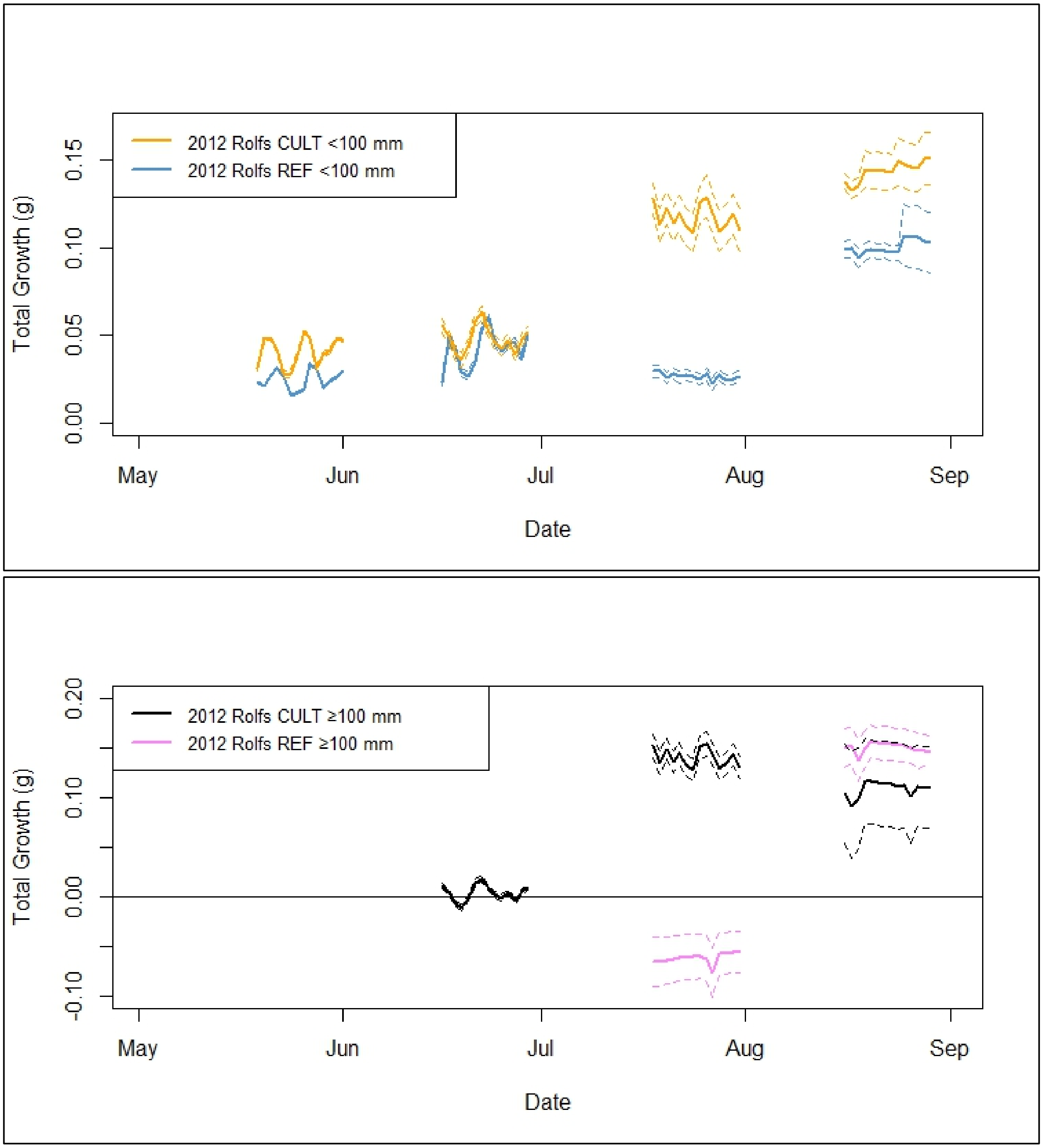

Figure 2.18. Wisconsin model projections for individual total growth of the two modeled size classes of sculpin at Rolfs cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

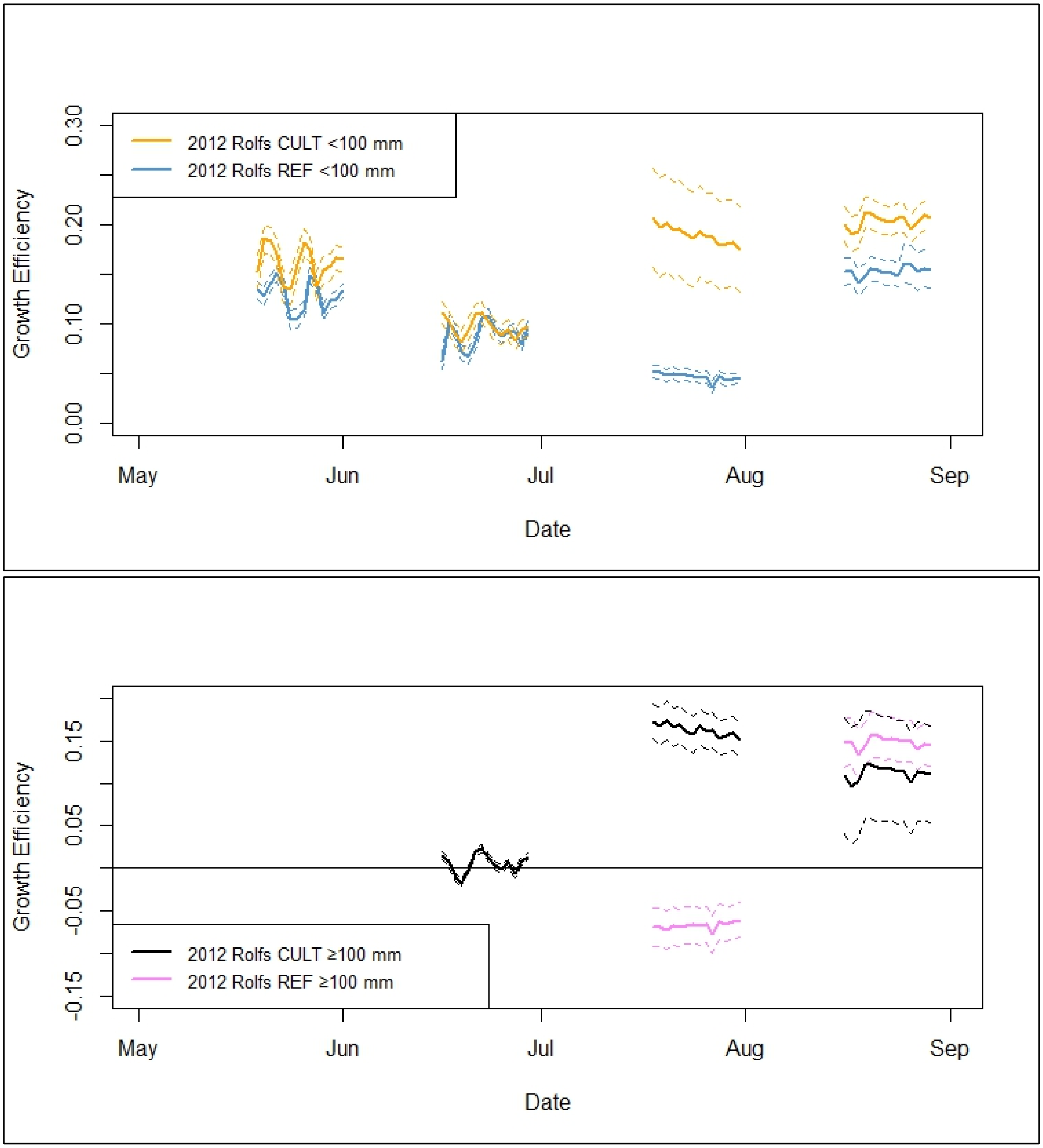

Figure 2.19. Wisconsin model projections for growth efficiency of the two modeled size classes of sculpin at Rolfs cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

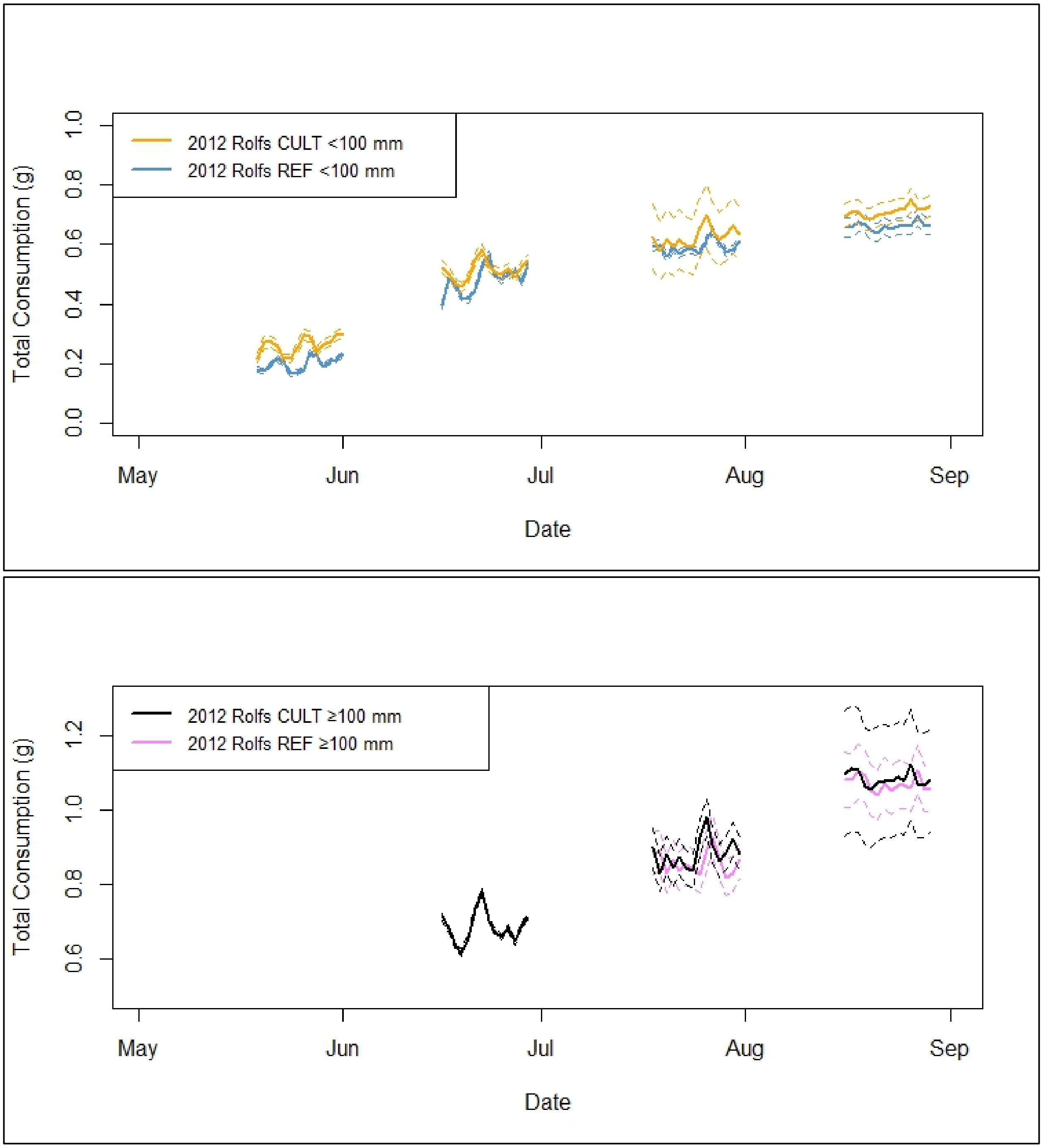

Figure 2.20. Wisconsin model projections for individual total consumption of the two modeled size classes of sculpin at Rolfs cultured and reference plots in 2012. Solid lines: mean projected value, dashed lines: 95% confidence intervals.

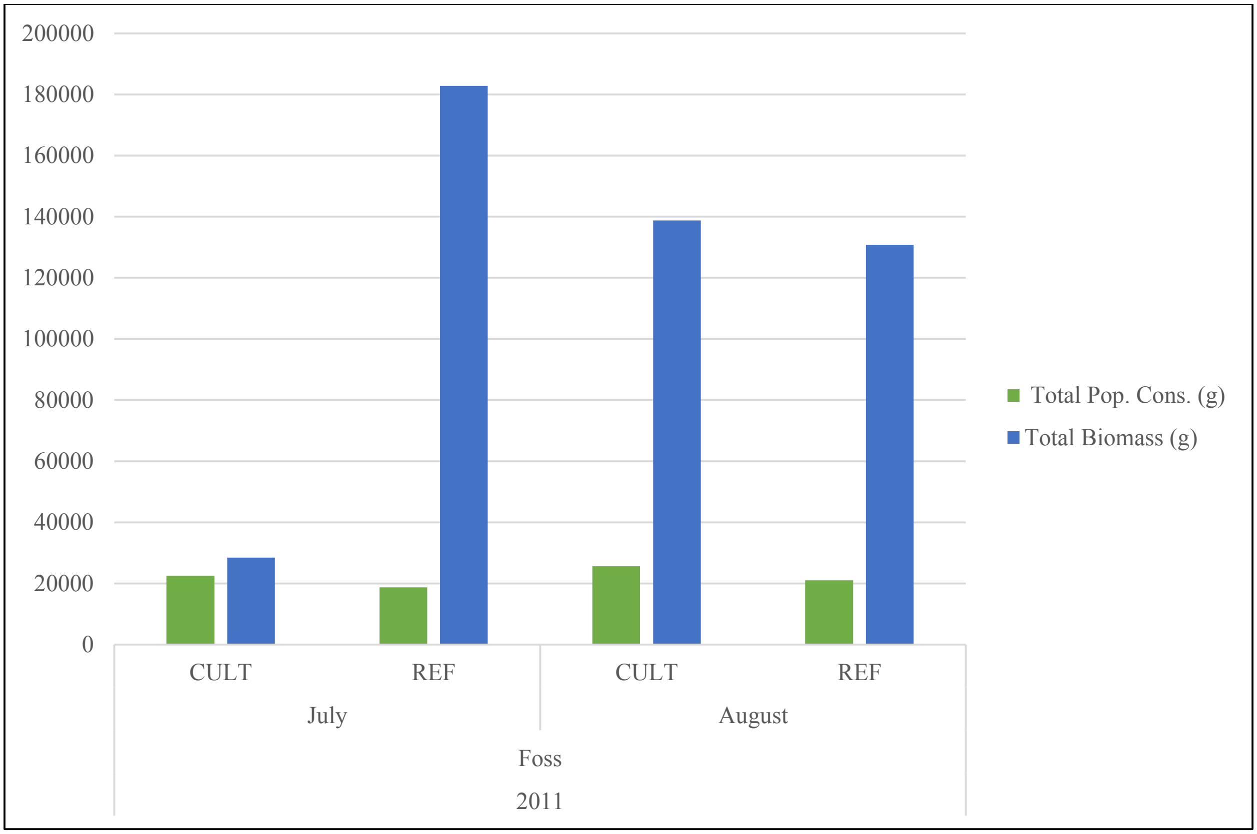

Figure 2.21. Total estimated consumption of a population of 1000 sculpin and total estimated prey biomass of a 5000 m^2 plot at Foss in 2011.

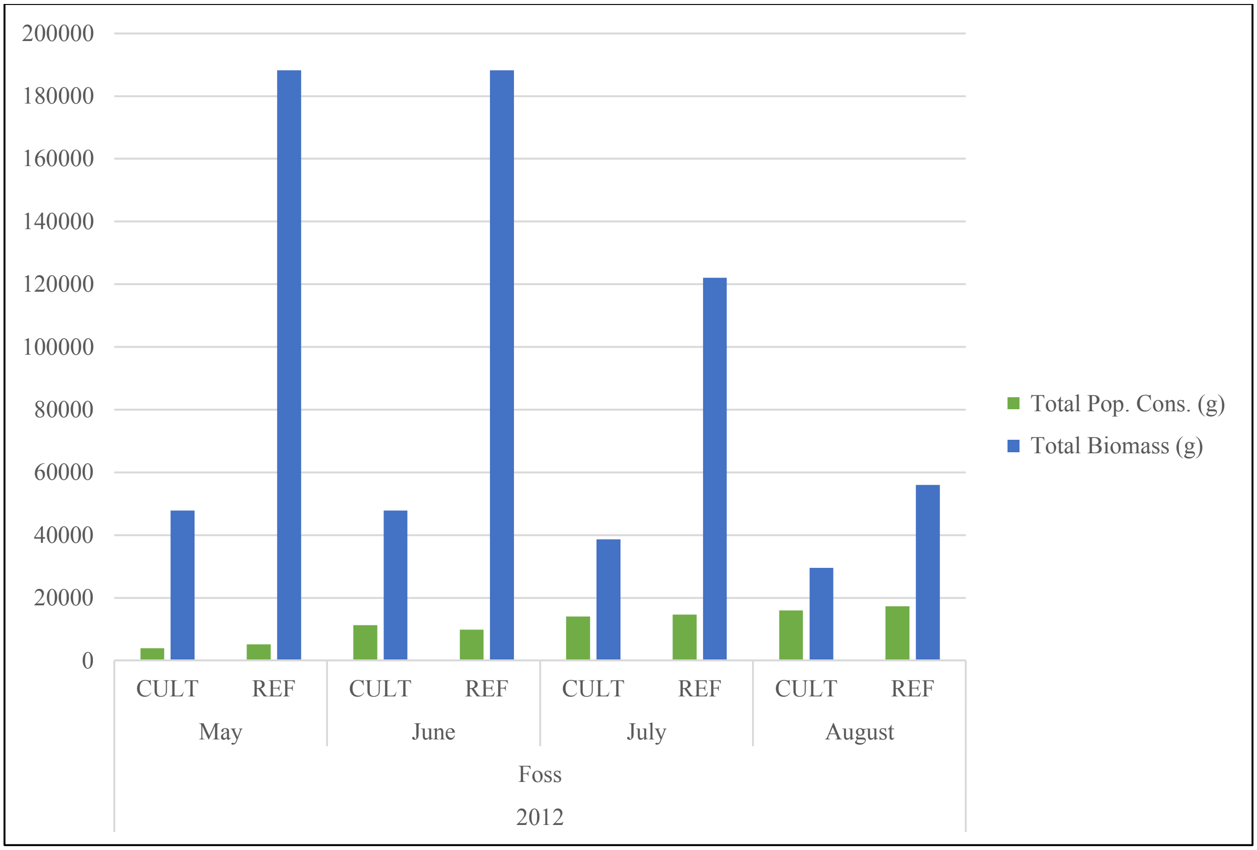

Figure 2.22. Total estimated consumption of a population of 1000 sculpin and total estimated prey biomass of a 5000 m^2 plot at Foss in 2012.

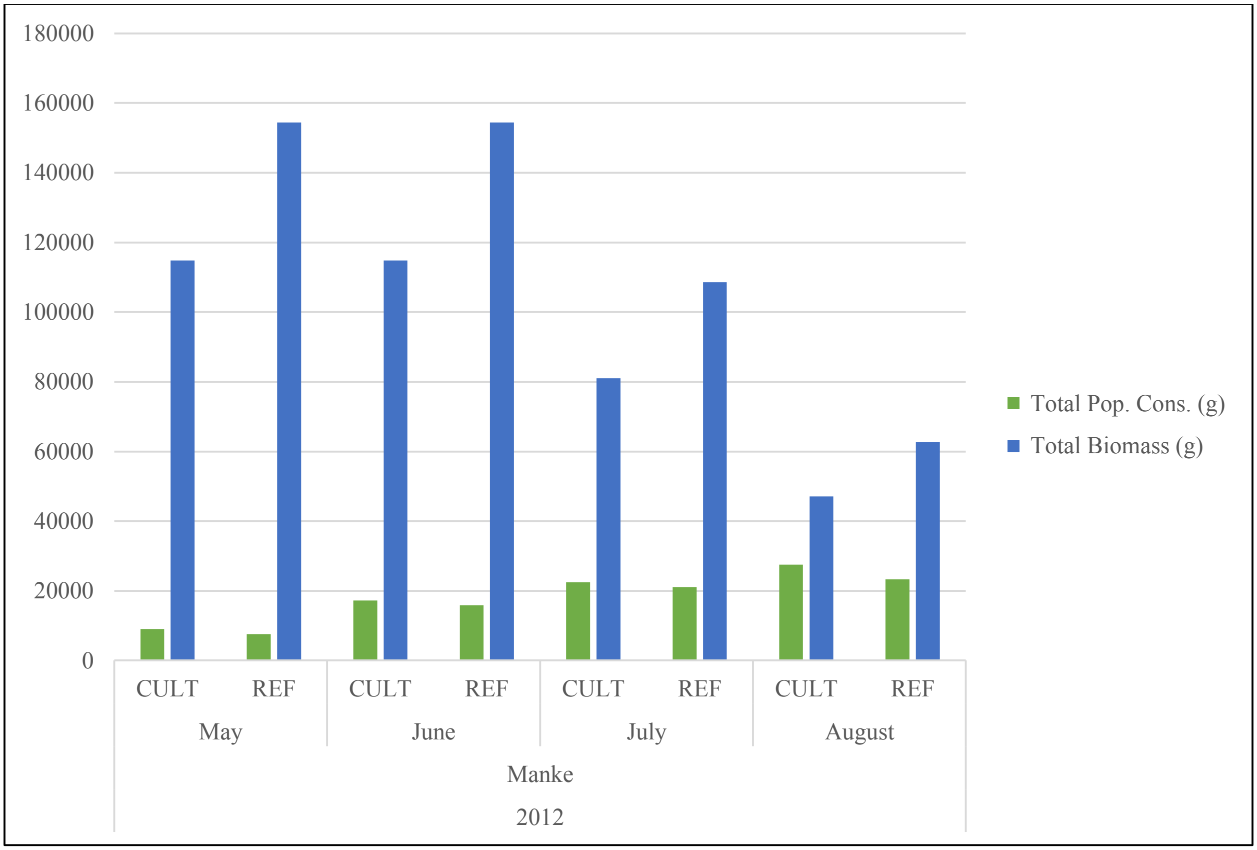

Figure 2.23. Total estimated consumption of a population of 1000 sculpin and total estimated prey biomass of a 5000 m^2 plot at Manke in 2012.

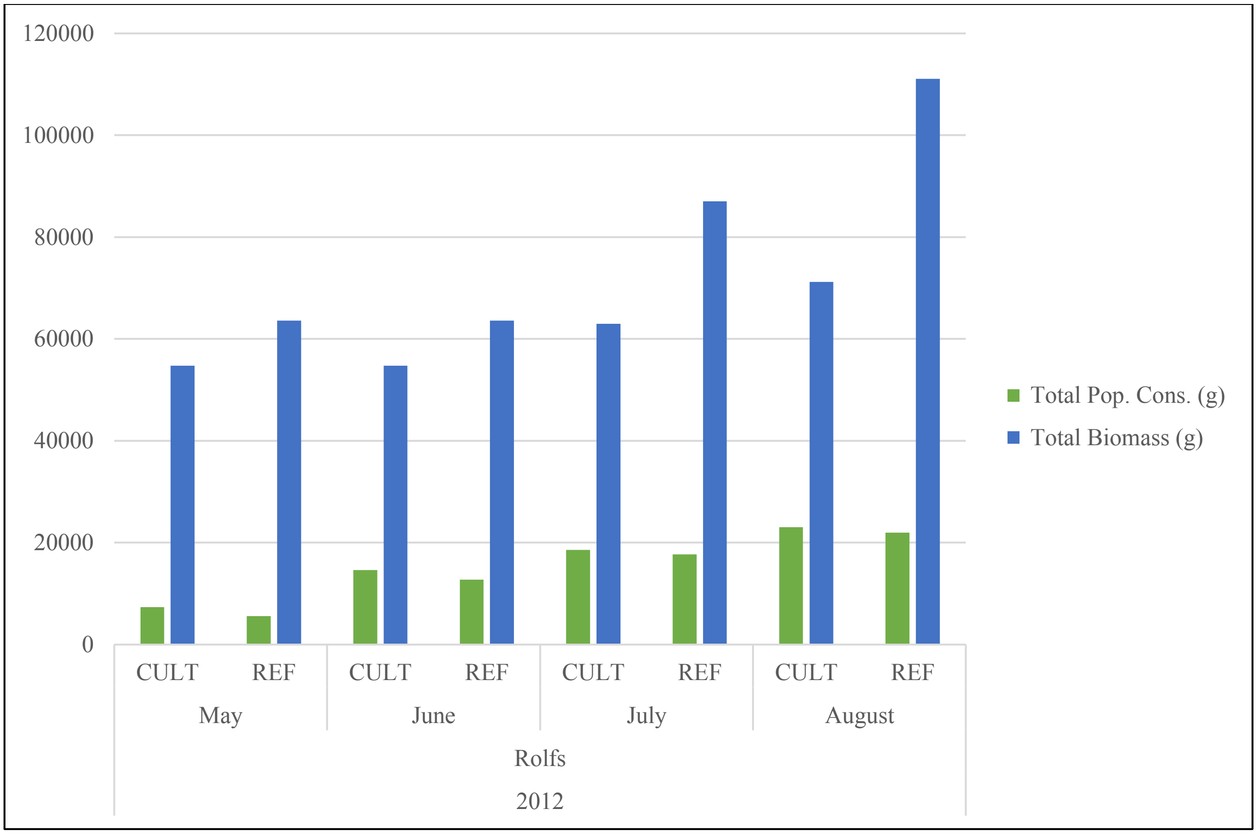

Figure 2.24. Total estimated consumption of a population of 1000 sculpin and total estimated prey biomass of a 5000 m^2 plot at Rolfs in 2012.

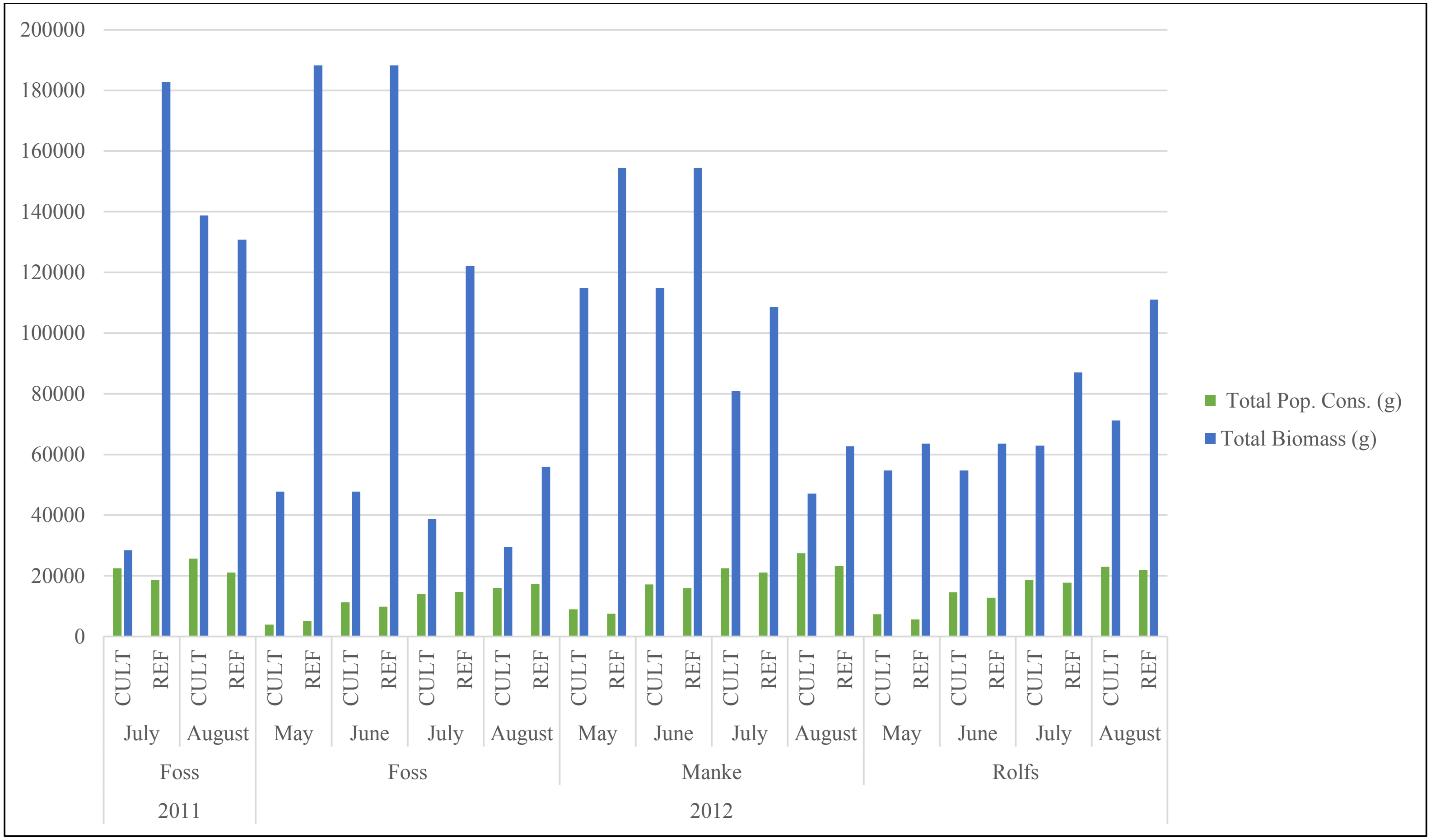

Figure 2.25. Total estimated consumption of a population of 1000 sculpin and total estimated prey biomass of a 5000 m^2 plot for all sites in 2011 and 2012.

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