

GROWING GOOSENECKS: A STUDY ON THE GROWTH AND BIOENERGETICS
OF *POLLIPICES POLYMERUS* IN AQUACULTURE

by

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THESIS ABSTRACT

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Title: Growing Goosenecks: A study on the growth and bioenergetics of *Pollicipes polymerus* in aquaculture

Gooseneck Barnacles are a delicacy in Spain and Portugal and a species harvested for subsistence or commercial fishing across their global range. They are ubiquitous on the Oregon coastline and grow in dense aggregation in the intertidal zone. Reproductive biology of the species makes them particularly susceptible to overfishing, and in the interest of sustainability, aquaculture was explored as one option to supply a commercial product without impacting local ecological communities. A novel aquaculture system was developed and tested that caters to the unique feeding behavior of *Pollicipes polymerus*. Modified feeds of bio-enhanced rotifers and a blend of up-cycled commercial fish byproduct were administered to barnacles of three different size classes. Growth and propogule potential were tracked in the culture tanks. In addition, a separate experiment was performed to investigate various biometrics associated with feed efficiencies and nutritive content to assess the overall sustainability of this aquaculture system.

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CHAPTER I
INTRODUCTION AND BACKGROUND

Taxonomy, Biology, Ecology

Gooseneck barnacles inhabit the west coasts of North America, South America, Europe, and Africa. This global distribution spans both tropical and temperate oceans, and encompasses four species: *Pollicipes pollicipes*, *P. polymerus*, *P. elegans*, and *P. caboverdensis* (Sousa et al. 2013). All species inhabit rocky coastline in the wave-exposed intertidal zones and all are of economic importance for food on their respective coasts. *P. pollicipes* is perhaps the most widely utilized species, as it is considered a delicacy in the southern portion of its range on the Iberian Peninsula. However, this Atlantic population extends from Brittany on the southwest English coast to Dakar, Senegal. *P. caboverdensis*, until recently thought to be a subpopulation of *P. pollicipes*, is found exclusively on the shores of the Cape Verde islands off the coast of Senegal. Pacific species include *P. polymerus*, found from the southern coast of Alaska to the Baja Peninsula in Mexico, and *P. elegans*, the southern species found from Baja to Peru (Fernandes et al. 2010). The four species share all major morphological characteristics with differences arising in the number of carinal plates as well as the color of the rostral aperture (Barnes 1996).

Slight morphological differences aside, all species fall under the broader sub-class Theocostraca, infraclass Cirripedia. As barnacles they are sessile suspension feeders classified by having two distinct swimming larval stages, the nauplius and the cyprid (Lewis 1975). Order Pedunculata attaches to substrate by a long muscular stalk, known as

the peduncle, distinguishing it from the acorn barnacles. The capitulum contains the rest of the body, including feeding appendages, gut, and reproductive organs. Six pairs of thoracic appendages constitute the major feeding structures, which extend through the shell aperture. The capitulum is girded by five calcareous plates that provide protection from predation and manual abrasion by waves, with progressively smaller scales approaching the junction of the peduncle. The anterior and posterior plates, the rostrum and carina, are the most distinctive and uniform between individuals and are therefore most commonly measured in growth studies.

The gooseneck barnacle is a simultaneous hermaphrodite and ovaries positioned in the mantle cavity release eggs, which form into egg lamellae masses in the mantle cavity (Barnes & Reese 1960). Sperm is typically exchanged along the extensible penis from one individual to the mantle cavity of a neighboring individual, where egg lamellae are fertilized and continue to brood until larval release (Lewis & Chia 1981a). There is only one case in the literature for self-fertilization in *Pollicipes*, a study based on genetics analysis, which also claims that *Pollicipes* have the potential to broadcast spawn (Barazandeh et al. 2013). Any individual that is farther than 20 cm from its nearest neighbor is therefore considered in the literature unable to reproduce (Cruz & Hawkins 1998).

While breeding season is heavily influenced by various environmental factors such as temperature, wave action, food supply, and salinity, reproduction peaks between April and October in the Northern hemisphere (Lewis & Chia 1981a). During this peak season, eggs are brooded within the mantle cavity for anywhere between 21 and 30 days before they hatch into larvae and are released into the water column. Planktonic nauplii

larvae undergo a series of molts, each successively larger with more advanced appendages and feeding behavior. After growing through naupliar stages I-VI over the course of about 30 days, they become non-feeding cyprids.

The cyprid of *Pollicipes* does not possess the nutrient-rich oil reserve typical of almost all other Cirripede cyprids, suggesting a short cyprid phase and rapid settlement (Lewis 1975). *Pollicipes* is a specialist settler; cyprids exhibit a high degree of conspecific affinity and almost exclusively settle on the peduncles of adult goosenecks (Barnes & Reese 1960). Experiments in the lab and in the field indicate that cyprids attach to conspecific adults at the capitulo-peduncular junction by secreting a bonding adhesive from their sensory antennules (Helms 2004). The exact settlement cue is unknown, however, researchers working with acorn barnacles, order Sessilia, have identified certain glycoproteins with amino acid compositions close to that of actin that are commonly associated with arthropod cuticle as factors that induce settlement (Hadfield & Paul 2001). Actin is responsible for various types of cell movement and participates in more protein-protein interactions than any other protein, making it a critical player in most cellular functions. While the molecular composition of *Pollicipes* growth zones remain uncharacterized, it is likely that actin-like glycoproteins are involved, and therefore would also play a role in gregarious settlement.

In addition to the possibility of a chemical settlement cue, cyprids are likely attracted to the pattern of calcareous scales on the stalk, which are well suited as landing and establishment sites for the cyprid larvae (Lewis, 1975). Once cyprids settle onto the peduncle they are very well camouflaged amongst the scales, which may provide a respite from predation. This highly ramified surface, together with chemicals at the

growth zone, may be the keys to cyprids' gregarious settlement. Previous experiments with *Pollicipes pollicipes* cyprids have introduced a variety of artificial substrata intended to mimic live adult peduncle with limited success (Franco 2014). I am unaware of any published work on the chemosensory coupling between *Pollicipes* cyprids and adults.

Gooseneck barnacles can form dense, carpeting aggregations in the low intertidal and distinct rosette-shaped clusters in the mid-high intertidal (Hoffman 1989). Their patchy, heterogeneous distribution may be attributed to the inconsistent habitat suitability at small spatial scales in conjunction with unique reproductive biology. Habitat suitability is dictated by competition with other intertidal organisms, food availability and environmental conditions that facilitate food capture. These barnacles are specially adapted to wave-pounded rocky coastlines where waves deliver food particles into their passive cirral net. the smallest juveniles between 1 and 7 mm Rostral-carinal (RC) length rhythmically beat their cirral fan to actively capture phytoplankton and small zooplankton in the water column (Hui 1983). Once they have grown past 7 mm RC length, feeding behavior shifts from active beating to passive extension of cirral fans that capture particles delivered by wave action.

In barnacles of any age, maxillipedes transfer food from larger cirri to the mouthparts, or can function as a direct filter for feeding on micro-particles (Anderson & Southward 1987). It is assumed that recently settled juveniles actively beat their feeding appendages to increase competitive advantage within a clump, where they must capture food alongside adults and likely other co-occurring intertidal filter feeders (Hoffman 1989). Beating also serves to ventilate the mantle cavity and maintain internal temperatures especially during egg-brooding (Anderson & Southward 1987). Insufficient

water motion or undesirable plankton quality in the nearshore could differentially affect aggregations that are mere meters away from each other, contributing to their patchiness (Barnes & Reese 1960) . The clumping phenomenon might also be explained by the fact that aggregations provide a benefit for reproduction, since cyprids preferentially settle on adults and clumps provide readily available, abundant substrate (Bidegain et al. 2017). Furthermore, as simultaneous hermaphrodites, their gregarious population structure provides the physical proximity necessary for successful fertilization. The largest and oldest barnacles are in the center of clumps, surrounded by smaller and younger individuals. The barnacles within a clump are able to exchange gametes with neighbors up to 11 cm away (Barnes 1965). The previously-mentioned affinity for conspecifics is another life history process that is linked to the high density of clumps in the intertidal. In the cases where aggregation is hindered by competition, predation, or human activity, local populations would be weakened by decreased rates of gamete exchange and larval settlement.

Economic Interest in Gooseneck Barnacles

A delicacy in Spain and Portugal and somewhat of a novelty seafood elsewhere, these sessile crustaceans are easily overexploited and historically have not fared well under intense fishing pressure. The southern populations of *Pollicipes pollicipes*, colloquially known as “percebes” in Spanish and Portuguese cuisine, are the most economically important resource from the rocky intertidal of the Iberian peninsula, with a market price as high as \$65 euros/kilo and approximately 196,000 metric tons harvested annually for commercial sale (Macho et al. 2010). After local stocks were heavily

depleted in the 1970s, Spanish and Portuguese market demand was met through importation from other countries like France and Morocco (Macho et al. 2010). In spite of some attempts to institute seasonal closures and catch limits, relatively unregulated exploitation continued on the Iberian peninsula until the early 1990's, when fishery authorities implemented a co-management regulatory scheme in attempt to halt precipitous stock depletions (Molares & Freire 2003). Within this regulatory structure, governments agencies shared responsibility for the percebes resource with local "cofradias", loosely organized groups of barnacle fishermen who were allowed to continue fishing under a TURF (Territorial User Rights for Fishing) regime (Rivera et al. 2016). Strong community buy-in enabled this system to expand to the point that there are over 60 cofradias located on the Galician coast now, and they regulate gooseneck harvest by only allowing permitted fishermen who are a member of the local cofradias to fish and sell their catch (García de la Fuente et al. 2013). Each cofradia decides for itself on regulations like number of fishers allowed, Individual Catch Quotas, daily maximum limits, and area or seasonal closures (Borja et al. 2006). Stocks on the Iberian peninsula are nowhere near their natural estimated densities however many local populations have responded positively to recent sustainability efforts and are stabilized and able to support the current level of harvest (Rivera et al. 2016). The decline of populations under harvest pressure from coastal communities is not surprising, considering life history constraints, the highly selective settlement on conspecific adults, which, when removed, also remove subsequent cohorts of juveniles.

While the Iberian coastline is host to the largest and most profitable gooseneck barnacle fishery, it is not the only one in the world. There was interest in opening a

fishery off the coast of British Columbia, Canada in the mid 1980's to supplement the European fishery, with the intent of selling live product to Basque markets (Bernard 1988). Invertebrate fisheries on the Canadian coast are sustainable by virtue of the relatively small harvest pressure and a management scheme that prioritizes marketing and product quality over landing volume (Jamieson & Campbell 1998). The commercial fishery experienced a set back in the early 1990s after overharvesting resulted in low stock recruitment for a couple years in a row, and since then the fishery has transitioned to being managed by the Nuu-chah-nulth a First Nations Tribe who had been harvesting them in a subsistence fishery for decades (Bigar 2017). The fishery is completely owned by the first Nations Tribe, who limit harvest to a few individuals within the community, and maintain natural stocks by restricting harvest.

Given the struggle to manage this complex resource in Europe, interest in a gooseneck fishery in Oregon has developed around a commitment to sustainability. Drawing on examples from historic successes and failures in fishery management, the gooseneck fishery can be crafted to avoid pitfalls plagued by other systems. Each fishery is of course unique and dependent on life history characteristics of individual species, however, there are general management schemes that can be applied broadly. Beverton and Holt (1958) developed influential models which have been widely applied to numerous global fisheries. These Dynamic-Pool and Surplus-Production fishery models for highly mobile demersal or pelagic resources provide a framework for population management that rely on the interdependence of four primary processes that determine the size and structure of marine fish populations: recruitment, growth, capture, and natural death (Beverton & Holt 1958). However, invertebrates with persistent spatial

structure and highly stratified population dynamics often do not conform well to fin-fish resource management models (Freire & García-Allut 2000). The gooseneck barnacle fishery, as such, defies these classic models due to hallmark characteristics including a complex life history strategy, gregarious settlement behavior resulting in heterogeneity at multiple spatial scales and local populations with limited dispersal connected at the regional level by planktonic larvae (Lipcius et al. 1997). The physical transport of larvae by ocean currents is critical to for maintaining healthy cohort proportions, but it perturbs stock-recruitment models that fisheries scientists and stock managers depend on.

Stock management often relies on catch restrictions by size or season as a tool to sustain populations. This is relatively easy for fish species where net mesh size automatically excludes certain age classes, or crabs which can be measured and thrown back into the ocean if they are too small or too large. Goosenecks, due to the nature of their gregariousness, are difficult to harvest by size class. As previously mentioned, the spatial structure of each aggregation is such that the largest barnacles (of high commercial value) are in the center, surrounded by densely packed, smaller ones. The entire clump must be scraped off the rock and harvested to procure the most desirable barnacles from each patch and once removed, they cannot reattach to the substrate and thus cannot live if returned to the ocean, such as one might throw a crab back if it does not meet the size requirements. The natural bonding adhesive that *Pollicipes* secretes from a specialized duct running the length of the peduncle is produced slowly enough that instantaneous reattachment to the substrate is impossible. Artificial reattachment such as gluing barnacles directly to rocks or installing plates that have been pre-seeded

with juveniles, is restricted by the severity of the typical gooseneck environment, where heavy wave action quickly erodes all but the strongest adhesives.

Sustainable *Pollicipes* Aquaculture

An alternative option to natural harvest for procuring a commercial seafood product is aquaculture. Seafood is one of the most important sources of both protein and nutrients for many communities, yet production from wild capture fisheries has slowed. In contrast, aquaculture is the world's fastest-growing sector of food production and now supplies over half of all seafood consumed globally (Liu et al. 2018). Despite significant contributions to food security in numerous countries, aquaculture has gained notoriety over the past two decades as an environmental disaster and destructive force, whether because of point source pollution, unwanted genetic infiltration to natural populations, reduction of wild populations as fish-feed, or massive die-offs associated with antibiotic-resistant microbes (Rico et al. 2017). As with the majority of industry, the balance between economy and the environment heavily favors economic interests, often at the detriment of the surrounding ecologies. However, these large scale, semi-intensive and intensive aquaculture operations are not the only option for cultivating marine organisms. The gooseneck barnacle, which is relatively incompatible with a high-pressure harvest fishery, has the potential to be a successful aquaculture species and adaptable to grow-out in culture. With mechanistic alterations that cater to specific feeding behaviors and preferred environmental parameters, *Pollicipes* culture could avoid the major pitfalls and environmental concerns associated with most commercial operations.

There are several factors that make aquaculture an enticing consideration for *P. polymerus*, including the possibility of a high end “delicacy” market in America to mimic the one in Europe, the fact that harvest activity is dangerous and dependent on tidal conditions, and the presence of biological life history traits that are inherently incompatible with current methods of harvest. Commercial aquaculture of barnacles is a yet-unexplored field of study. Up to this point, research into aquaculture of *Pollicipes* has been purely academic and there is very little in the way of optimized diets, feeding regimes, bioenergetics, food delivery methods, or tank conditions for stalked barnacles. These conditions are well established for other commercial mariculture species and offer a standardized baseline for grow-out operations.

Extensive research has been done in pursuit of perfecting crustacean aquaculture systems for commercial taxa such as lobster, crab, and shrimp, most of which generally fall into classes Branchiopoda and Malacostraca (Wickins & Lee 2002). Maxillopoda, which includes all barnacles, has garnered little commercial interest. While *Pollicipes* life history strategy and general biology is vastly different from most farmed crustacean, the baseline physiology is similar enough that comparisons can be made across culture systems, particularly where nutrition is concerned. Successful aquaculture hinges upon balancing nutrition and growth against cost and waste. Various considerations must be made for individual species’ feeding strategies, differences in nutritional requirements across life stages, and interactions with their physical environment.

The dietary requirements of crustaceans differ in several ways from other more commonly farmed marine taxa such as fish and molluscs, but remain similar in enough ways to allow for some generalizations across all crustaceans (Wickins & Lee 2002). In

general, crustaceans require a higher protein ratio than other aquatic invertebrates, perhaps a byproduct of a natural environment that is rich in varied protein sources (Holme et al. 2009). While it is impossible to mimic that vast diversity in an aquaculture setting, diets that provide a complementary set of essential nutrients and proteins should ultimately result in the healthiest and fastest growing organisms. In the long term, protein is the single most expensive input for any aquaculture set up and therefore protein substitutes or diets that can be effective with a lower protein ratio are preferred. Crab farmers have found that protein content can be lowered in culture situations as long as the ten essential fatty acids are present in the feed (Anh et al. 2011). These ten essential amino acids are the same for most farmed crustaceans: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, although the ideal quantitative distribution for different species has yet to be determined (Wickins & Lee 2002).

Challenges in aquaculture span the entire life cycle of *Pollicipes*. Fertilized egg lamellae are present in mature adults from late spring through early fall, but *in-vitro* rearing would then require conditions that mimic the mantle cavity for normal development to occur (Franco 2014). Hatching can be induced via manual abrasion of the egg cluster or by periodic inundation cycles that replicate low and high tides (Franco 2014). Larvae, once identified in the plankton, could be collected from coastal waters at peak periods between spring and early fall, but inducing settlement and metamorphosis in *Pollicipes* cyprids in the lab has historically proven more difficult than other Cirripedes such as *Balanus* or *Semibalanus* (Hadfield & Paul 2001). The major bottleneck arises from a gap in the knowledge pertaining to settlement cues; cyprids preferentially choose

live adult peduncle, but it is unclear whether that affinity arises from chemosensory attraction, associated bacterial biofilms, or a tactile surface that promotes adhesion.

Another challenge arises in optimizing growth conditions for adults. To feed, adult gooseneck barnacles extend their cirri, which expand like a net at a suitable angle for passive food capture (Crisp & Stubbings 1957). They periodically withdraw these biramous appendages into the capitulum cavity where food particles are scraped off by smaller, setous maxillopedes and transported to the mouth (Page 1983). This method of particle capture is well adapted to high wave energy rocky shelves where they are commonly found. However, introducing that much motion into an aquaculture system is expensive and could be a limiting factor if barnacle aquaculture is to be scaled up to produce commercially viable outputs.

Bioenergetics and Culture Efficiency

Metabolism and nutrient assimilation are key considerations in choosing formulated diets for aquaculture species (Seibel & Drazen 2007). Aside from protein, carbohydrates, and lipids, the macronutrients that are essential for growth and development, additional micronutrients, vitamins, and fatty acids contribute to a healthy diet profile and increased growth and reproductive efficiencies in lab. A formulated diet can be well balanced and contain all the essential dietary nutrients, but still not produce desired results if the various nutrients are not readily available (Lucas & Southgate 2012). The true nutritive value of a formulated diet therefore ultimately depends on the bioavailability of the ingredients, and not purely on diet composition (Teshima et al. 2000). In terms of desirability of commercial aquaculture species, one area in which

crustaceans excel is their feeding efficiency which is commonly quantified and reported as a Feed Conversion Ratio (FCR). FCR is an indicator used in all types of farming and husbandry and provides a standardized indication of how efficient a specific feed or feeding strategy can be (Boyd et al. 2007). The formula is relatively simple: $FCR = \text{feed input mass} / \text{animal biomass gain}$. The lower the number, the more efficient the feed system. By this measure, subsidized aquaculture and farmed chickens are similarly efficient at converting feed into animal biomass, and both are more efficient compared to farmed pigs and cattle (Hardy 2009). FCR does not account for variation in feed content, edible portion of an animal, or nutritional quality of the final product (Fry et al. 2018).

When properly standardized, FCR is one tool that aquaculture industries can use to compare across different scales and systems of production. Inaccuracies in this metric stem from the ways in which certain parameters are accounted for including mortality rate, waste estimation, population sampling method, and accuracy of calculating actual consumption rates (Tacon & Metian 2015). Despite these flaws, FCR remains a useful tool for comparing relative farming and dietary efficiencies across taxa and will be a useful tool for quantifying the success of gooseneck aquaculture techniques. FCR provides complimentary information to an otherwise growth-oriented aquaculture project.

Summary of Chapters

In Chapter I, I report on the experimental aquaculture system that was a novel development for this project and the associated findings on growth, diet, and recruitment. The aim of this experiment was to test both “intensive” and “extensive” aquaculture systems for gooseneck barnacles in Charleston, OR and find which associated conditions

and diet produced the highest growth rates at different life stages. Barnacles in the “intensive” experiment were fed either rotifers or proteinaceous tissue of fish and kept in constant turbulence. Barnacles in the “extensive” field experiment were deployed in the marina with no food subsidy other than whatever local plankton were available in the water column, over the two month course of the experiment.

In Chapter II, I report on Feed Conversion Ratios and other biometrics for gooseneck barnacles in an aquaculture setting. Adult barnacles have a fully developed gut and the complete set of digestive enzymes required for their mostly-carnivorous diets (Norton 1996). There is a high chance that adult barnacle will be able to consume and assimilate nutrients from a wider range of potential feeds. However, feed efficiency values do not exist for *Pollicipes polymerus* currently, so my second chapter will report on feed conversion ratios for barnacles consuming diets of rotifers and upcycled protein sourced from fish processing byproduct. Feed amounts and barnacle biomass were carefully tracked in order to place *Pollicipes polymerus* within the existing bio-energetics literature. I also examined protein retention and concentration in barnacle tissues and feed materials for comparison across other commercial seafood products.

CHAPTER II

MARICULTURE GROWTH

Introduction

As the future of wild harvest fisheries continues to tighten around collapse and mismanagement of stocks, aquaculture has risen to fill that space in the global market as a practical and sustainable alternative. Mariculture, spans a wide range of practices and production systems. Mariculture can be classified according to degree of commercialization (i.e., subsistence, artisanal, life-cycle specialization, or industrial; Lazard et al. 1991), distribution of product (i.e. protein remains within the community that harvested it or protein is exported; Martinez-Espinosa 1995) or, most commonly, mariculture is classified based on degree of sophistication of the technology, feed, and production system. Edwards & Demaine (1998) defined three tiers based on a set of industrial characters: “extensive” systems require no additional nutritional inputs beyond what is available in the given environment and depend on natural conditions for all culture needs. “Semi-intensive” systems also utilize natural food but productivity is artificially enhanced by manipulating fertilization/nutritional factors and use of supplemental feed at specific life stages. “Intensive systems” depend on formulated feeds with specified nutrition profiles and typically involve complicated infrastructure to support the production of cultures at artificially high densities. In addition feed, labor, capital, and management increase dramatically up each tier (Tacon & Metian 2015) as do waste production and potential for detrimental effects to the surrounding environment.

Relevant Biology

While the culture of marine predators, primarily fish, are considered intensive, culture of marine invertebrates typically falls in the extensive and semi-intensive categories, due to their generalist role in the natural trophic structure coupled with physiological requirements that are easier to satisfy relative to culture of fish. Almost all molluscan mariculture and some crustacean culture systems fall under this category, where organisms are placed in natural environments and grown to market size with minimal inputs. Sessile, benthic planktivorous feeders thrive in this type of system. One exception to the rule is *Pollicipes polymerus*, an intertidal stalked crustacean in subfamily Cirripedia.

Representatives of the genus are found on the west coasts of Europe, Africa, and the Americas and harvested and eaten by coastal communities throughout most of its global range (Borja et al. 2006). Gooseneck barnacles prefer exposed coastline where strong surges from wave motion deliver plankton to rocky crevices and ledges on which *Pollicipes* forms patchy but dense aggregations (Barnes & Reese 1960). Gooseneck barnacles are simultaneous hermaphrodites that do not self-fertilize and do not broadcast spawn (Cimberg 1981). The maximum distance of gamete exchange is typically no more than the maximum extensible length of the penis, which is contained in the capitulum, although some evidence for limited broadcast spawning does exist (Barazandeh et al. 2013). The capitulum cavity also contains egg lamellae, where eggs are brooded post-fertilization until naupliar larval forms within each egg are released (Cruz & Hawkins 1998). For this reason, gregarious behavior and patchy distribution of populations is likely an evolutionary consequence of reproductive biology that depends on small

distances between conspecifics (Strathmann 1987). It is also likely that adult survival is enhanced by clumping, because it fortifies populations against competition by mussels.

Being a member of Cirripedia, the gooseneck barnacle has two distinct pelagic larval stages, the feeding nauplius and the non-feeding cyprid. The *Pollicipes* cyprid exhibits a high degree of affinity and almost exclusively settles on the peduncle of adults (Barnes & Reese 1960, Heip 1975, Sousa et al. 2013). Inducing settlement on any substrate other than live peduncle sheath has had limited success (Franco 2014). Cyprids use specialized antennules to attach to the top of the peduncle. As juveniles grow, they move down the length of the stalk before eventually attaching to the substrate to form concentric, rosette-shaped clusters (Helms 2004). The attachment to hard substrate is permanent, marking the final transition to a benthic, sessile existence. The upright structure of the adult peduncle is maintained via internal haemolymph pressure that affords flexibility in response to mechanistic abrasion from waves but can be altered to expand or contract in response to environmental stressors (Barnes 1996). The peduncle consists of a column of muscle contained by a chitinous sheath and is the portion of the barnacle consumed by humans.

Harvest Potential and Existing Fisheries

The west coast of North America harbors stretches of coastline where gooseneck barnacles grow abundantly in the intertidal zone. This species is still absent from mainstream culinary culture. The largest barnacle fishery on the west coast is a small, community-regulated Nuu-Chuh-Nulth fishery off the coast of British Columbia that supplies all the barnacles for Spanish specialty cuisine shops across the United States (Gill 2015). The typical order size is 45-90 kg and the fishery CPUE (Catch Per Unit

Effort) is 9-15 kg* hr⁻¹ (Schiller 2015). At present, the fishery is very small, with only four groups of 2-3 individuals, each collecting barnacles from the 48 designated harvest rocks (Laurenne Schiller, pers. comm.). Given these ecological and biological parameters, only a very small portion of the total stock within the T'aaq-wiihak Fishing Area on the west coast of Vancouver Island is harvestable at any given time, and it is considered a highly sustainable fishery.

Other instances of commercial activity include individuals who harvest on commercial permits and sell to local communities, however, Oregon Department of Fish and Wildlife (ODFW) only allows harvest off of man-made structures such as jetties and breakwaters (ODFW 2016). This conservative management strategy arose in response to the current state of *Pollicipes* populations on the Iberian Peninsula. Following the trajectory of many marine resources, this delicacy has been overharvested to the point of population collapse in certain localized areas in Spain and Portugal (Otto 2013). Management in Europe has instituted a mitigation strategy based on zones of community jurisdiction and strong local controls and social buy-in (Bald et al. 2006). This scheme is effective for a group that shares a regional culture and depends almost entirely on the ocean for economic stability (Molares & Freire 2003). However, the situation in Oregon, where there is no tradition of harvesting goosenecks and the wild stock is still robust and healthy, remains open to a range of strategies that would contribute to a sustainable fishery. One such strategy is mariculture, which can provide a separate commercial product or supplement natural populations should harvest increase.

Gooseneck Barnacle Aquaculture in Oregon

The mariculture of *Pollicipes polymerus*, as described in this study, falls under the designation of “semi-intensive”. It is relatively low in its trophic position, does not spend energy moving around to capture prey, and cannot escape from culture systems. These characteristics would qualify *Pollicipes* for “extensive” mariculture if not for their unique feeding behavior that is dependent on heavy wave action, without which they do not extend their cirral feeding appendages (Hui 1983).. This aspect of gooseneck life history strategy significantly changes the approach to mariculture. The barnacles require a dynamic physical environment, which must be created in a lab culture set up. Tanks must provide adequate water flow to initiate feeding while still operating below a certain level of water use so that the system does not become too costly. Adding turbulence and water flow into a culture system is both expensive and difficult to standardize and affects food delivery methods and circulation logistics.

This study proposes a “semi-intensive” mariculture design that solves the turbulence problem, coupled with two experimental formulated feed treatments to maximize growth. In addition, an “extensive” system was tested in the field, where barnacles were placed in a natural environment with no modification. Each system offers benefits and drawbacks in the overall scheme of mariculture efficiency and potential for scaling up to a commercial-size operation.

Materials and Methods

Collection

Pollicipes polymerus individuals were collected from Drakes Point in South Cove, OR (43° 18' 10.242" N, 124° 23' 58.5816" W) on September 07 2017 at a morning low tide. Barnacles were collected with a metal spatula by scraping clumps off rocks. Entire clumps were harvested to minimize incidental mortality. In the lab, barnacles were sorted into size classes according to rostral carinal length (RC), the most widely used metric for tracking gooseneck growth. "Small" barnacles (3-8 mm RC), "medium" barnacles (10-15 mm RC) and "large" barnacles (17-25 mm RC) were separated and 128 individuals from each size class were chosen as representatives for the experiment. The peduncles of those chosen for aquaculture experiments were scraped clean of all visible cyprids and recently settled juveniles.

System construction

The basic concept and design of the aquaculture tanks and flow system were adapted from a previous experiment by Bingham *et al.* (2017). Tanks were constructed of 10 cm ID (inside diameter) sections of ABS (acrylonitrile butadiene styrene) pipe cut into 40 cm lengths and positioned vertically in the sea table so that each tank was a cylinder standing on its end. The distal end of each tube was attached to an acrylic sheet base that prevented leaks and provided stability. Each section of ABS cylinder was attached to its base with both super glue and silicone adhesive. Holes were drilled into the bottom of the ABS tube for inserting ports for air and water. The top of each tank was uncovered and open, allowing bubbled air to escape and water to overflow the side to ensure continued circulation. For each ABS cylinder, a section of ¾ in PVC pipe was

seeded with 8 barnacles and “planted” in the exact center. The central PVC rod was kept vertical by inserting it into $\frac{3}{4}$ in PVC end cap that was glued to the base of each tank. The central rod could be removed easily for each cleaning and measurement event and then replaced in position (Fig. 1). The diffuser at the bottom of each tank encircled the central rod and provided a vertical curtain of bubbles, which served the dual purpose of initiating cirral feeding behavior and suspending food particles for the duration of each feeding period. Air and water were delivered via a centralized manifold system, which utilized $2.05 \text{ L} \cdot \text{min}^{-1}$ water flow spread across 48 tanks and $2.58 \text{ m}^3 \cdot \text{hour}^{-1}$ of air supply at 75-80 PSI. All port connections and lengths of connecting hose were standardized to maintain equal delivery of air and water to all tanks. Central manifolds with adjustable ball valves leading to each tank aided in this process, however, flow rates between tanks were subject to variation of up to $\pm 0.34 \text{ L} \cdot \text{min}^{-1}$.

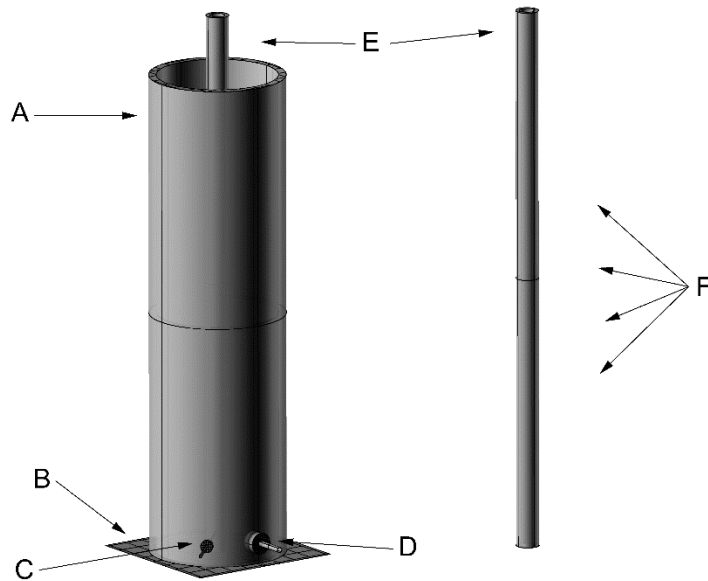


Figure 1. Mariculture tank design, constituted by (A) 1.5L ABS pipe tank, (B) stabilizing polyacrylic base, (C) air inflow port, attached to diffuser tubing coiled inside the tank and providing bubbles, (D) water inflow port, (E) inner column of $\frac{3}{4}$ in PVC to which 8 barnacles were attached (F).

An array of 48 vertical ABS tubes housed 2 different diet treatments and 3 different size classes, for a total of eight replicates of each combination of diet and size class (Fig. 2). The tanks were designed to mimic natural intertidal conditions while minimizing cost and maintaining ease of access for cleaning and growth monitoring. In the intertidal, barnacles are exposed to high flow rates and are also submerged for a significant portion of each day. Recreating these conditions in an experimental set up requires significant infrastructure. The literature up to this point indicated that barnacles require a minimum flow of $23 \text{ cm} * \text{s}^{-1}$ (Franco 2014) which is expensive to distribute across an array of experimental tanks and would inhibit scaling up to commercial levels. However, the water flow requirement is really a composite of two distinct features: exogenous mechanical stimulation to induce feeding behavior and a method of food delivery. In this study, air bubbles provided mechanical stimulation and the vertical nature of standing tubes provided a method of food suspension and delivery that allowed for a much lower water flow than previously thought possible.

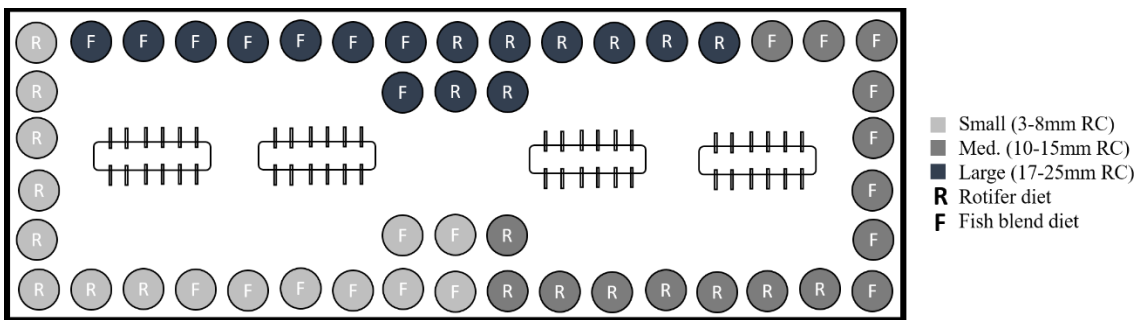


Figure 2. Schematic of tanks housed in one open sea table that constituted the infrastructure of the “semi-intensive” system of Exp. 1. Two sets of four manifolds in center provided equal air and water supply to all tanks.

Barnacles were attached by their bases with marine-rated superglue to the central PVC rod. Each barnacle was oriented with the rostrum facing downward so that cirral nets, when extended, would open into the direction of water and air flow. Eight barnacles total were introduced per tank, glued to opposite sides of the length of the rod. The diffuser coil tubing at the bottom was equidistant from the central rod and outside wall so that bubbles were directed into the space occupied by extended barnacle cirri. Vertical water velocity within each tank was $0.31 \text{ cm} * \text{sec}^{-1}$. Temperature fluctuated between 11-16.1° C based on ocean conditions.

Experiment 1: “Semi-intensive” Diet Manipulation

To investigate the effects of diet on growth in various life stages, diet manipulations were nested within size classes so that each of the three size classes was subjected to both diet treatments. This experiment was run from September 08 2017 to December 20 2017. Barnacle Rostral Carinal (RC) length in mm was used as a proxy for growth and measured each week using electronic calipers. Tanks were cleaned every other week to prevent accumulation of organic waste. Peduncles were thoroughly inspected for new recruits each week and settlers could continue growing. The two formulated feeds tested were live rotifers and a blend of fish-based protein. To ensure that food was retained in the system for an adequate amount of time to allow for feeding, water flow was shut off for 6 hours per day when as food was added to each tank. Aeration kept food particles suspended, and water circulation was reestablished at the end of the feed period. Prior to the start of the experiment, tank circulation was examined in a transparent demo tank (including a central rod) using microscopic free floating pieces of glitter plastic and verify even distribution of introduced food particles with the given air

flow design. The small flakes of plastic were an appropriate size (100-400 μm diameter), were highly visible, and simulated the size of rotifers and fish guts. Circulation was observed every ten minutes for a period of one hour to document potential changes over time. This was a strictly observational proof-of-concept. Live barnacles were not used. One potential source of noise in the data was related to an extenuating circumstance during week 5 which resulted in water flow being cut to half its previous flow for the remainder of the experiment, decreasing overall flow to $0.98 \text{ L} \cdot \text{min}^{-1}$.

Rotifers (*Brachionus plicatilis*) were bought as cysts from Florida Aqua Farms and reared using culture procedures outlined in the Hoff and Snell Plankton Culture manual (1987). Rotifers were fed a diet of Roti-Rich© fortified yeast in combination with concentrated *Nannochloropsis* algae. Rotifers are a common prey item utilized in aquaculture due to their large size, slow swimming speed, and high nutritive content (Dhert et al. 2001). Previous research on gooseneck barnacle feeding behavior and bioenergetics established a clearing rate of 81 rotifers per hour after a 24hr period of starvation (Norton 1996). Due to the constraint of only 6 hours of guaranteed feeding, 3.73×10^5 rotifers per day is the ideal harvest ($81 \text{ rot barnacle}^{-1} \text{ hr}^{-1} \times 24 \text{ hrs.} \times 8 \text{ barnacles/tank} \times 24 \text{ tanks}$). However due to instability in my rotifer cultures, the final feed density was 9.2×10^4 rotifers/day, which equals 80 rotifers per barnacles per hour for the 6 hours of guaranteed feeding time.

The other experimental diet was seafood industrial byproduct and waste material, collected from fish carcass cleaning stations at the Charleston Marina and a fish processing warehouse at Chucks Seafood Company in Charleston, OR. Fish heads and discarded body material were brought back to lab and any remaining muscle tissue,

internal organs, viscera, or other salvageable tissues were collected, dehydrated, and then ground to a particle size closely approximating a rotifer (100-400 μm particle⁻¹). A common food dehydrator was used to desiccate the tissues and then the dried pieces were ground using a Thomas Scientific Wiley Mill. All fish tissue was utilized equally apart from certain fatty tissues (i.e. fish liver) that proved too sticky for the grinder. This powder was homogenized, emulsified and fed to barnacles during the same time interval as rotifers. Upcycled fish protein was explored as a diet because it is a cheap source of high-quality protein and provides a use for industrial waste (Stevens et al. 2018).

Experiment 2: “Extensive” Docks Experiment

An “extensive” field experiment was conducted to test whether a significantly lower ambient flow would yield survival and growth of barnacles in ABS cylindrical tanks. Six tanks from Exp. 1 adapted for field use were deployed in the inner boat basin on I dock from March 29, 2018 to June 14, 2018 (Fig. 3). A mesh grate affixed to the bottom of each tank replaced the acrylic panel used in Exp. 1. A length of rope was passed through the mesh grate and then to a brick to counteract the buoyancy of the ABS pipe. Each rope was threaded through the hollow PVC rod upon which barnacles were glued, and in this way the main integrity of the structure was preserved from Experiment 1 to Experiment 2 (Fig. 4).

Each tank was equipped with a ring of diffuser tubing at the bottom to provide vertical air flow and turbulence. Air power was supplied by small aquarium pumps with two valves that utilize 1200cc for a total of $0.6 \text{ L} * \text{s}^{-1}$ per valve. Each pump supplied air for two tanks. Due to the pressure at six meters, air flow out of the diffuser tubing was significantly diminished compared to the air flow in the tanks of Experiment 1. The

active fouling community of the Charleston docks rendered the diffuser tubing inert within the first two weeks and the remainder of the experiment was run with no supplemental air. Due to the position of the diffuser tubing inside each tank, assessing the full coverage of the fouling community was difficult, and systematic scraping had limited success. Average Flow at I dock measured during consecutive incoming and outgoing tides was between 0.0 and 0.02m/s. Water flow measured with a Flo-mate model 2000 (Marsh McBirney) at 6m depth.

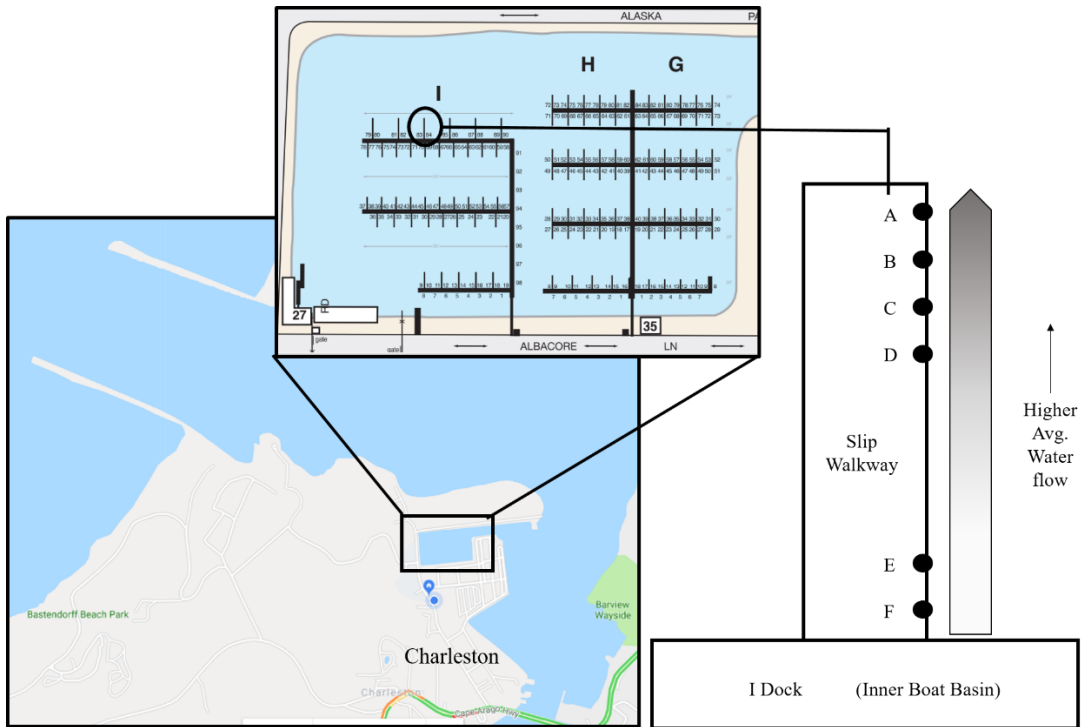


Figure 3. Dock Experiment location and orientation. Tanks A-F were deployed along the edge of a boat slip still in use, necessitating the large gap between D and E. Gradient represents approximate waterflow differences between the inner slip and outer slip.

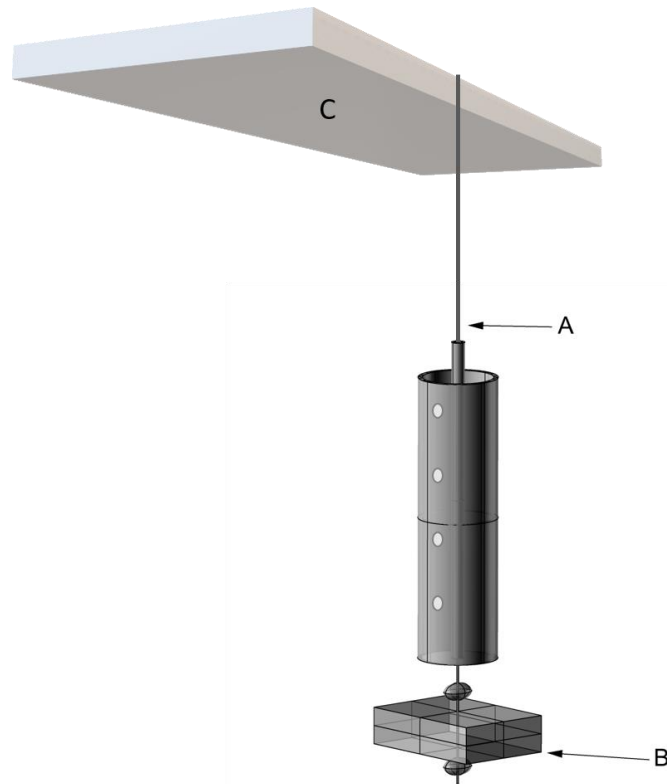


Figure 4. “Extensive” dock experiment set up, consisting of (A) rope threaded through central pole and tied to (B) brick for counteracting buoyancy of ABS tubing outer shell. Holes were drilled into sides of ABS outer shell to increase water exchange. The entire structure was suspended 2m below a boat slip walkway on I dock (C). Barnacles were glued to the central pole, similar to Exp. 1.

The internal PVC rod, seeded with 12 (new) individual *Pollicipes*, was also threaded through the rope. Each of six tanks was hanging off one side of a public slip and deployed at 2m below the surface. Holes were drilled into the sides of the ABS housing to allow increased water flow. There was no additional air or artificial water circulation, as bubbling air proved logistically impractical. Air flow was too low to prevent settlement upon diffuser tubing and members of the fouling community quickly took advantage of new structures introduced into the boat basin.

Data Analysis

All data was analyzed in R (R Core Team 2013). Before comparing treatments, treatment datasets were tested for normality (Shapiro-Wilkes Test, $P > 0.05$) and all potentially confounding variables were tested for rates of non-variance. Variables that were tested include comparisons before and after the change in flow regime and the effect of individual manifolds which mediated flow and therefore could affect delivery of cyprids as well as clearance rates of waste in each tank. None was a significant source of variance (T-test, $P > 0.05$). Barnacles were grouped according to diet (rotifer, fish) and size (small, medium, large). All treatments exhibit normality after being log-transformed. Raw growth data was log transformed in order to make the patterns in the data more interpretable. All statistics were performed on log-transformed data. All data was back-transformed before being graphed.

Proportional growth was calculated in addition to absolute growth. Absolute growth is an important metric for aquaculture but can often obscure certain biological aspects which are inherently different between size classes. For instance, Rostral-Carinal growth rates in *Pollicipes* are logarithmic, with significant increase in RC length in the first year followed by slower growth. The barnacles in each size class were introduced into the culture system at different points in their life and varying allometries and energy allocations at these different stages will affect overall growth. Absolute growth obscures these differences. To find a relative change in size over time, proportional growth was calculated as $(RC_f - RC_i) / RC_i$, where RC_f is final RC measurements and RC_i is initial RC measurement for each individual barnacle.

Results

Experiment 1.1 “Semi-intensive” Diet manipulation

The manifold arrays (of which there were four) did not a significant factor for differences in growth (ANOVA, $F = 1.09$, $df = 3$, $P=0.35$). Similarly, the change in water flow did not affect growth. When comparing growth across size classes, large barnacles demonstrated a mean growth of 1.53 mm RC over the course of 8 weeks, the highest of the three treatments ($0.765 \text{ mm RC} \cdot \text{month}^{-1}$, 0.4 SD) and there was a significant difference in growth rates between size classes (M-ANOVA, $F = 24.7$, $df = 2$, $P=1.03 \times 10^{-10}$). The difference in absolute growth between the two diets within the same size class was significant for medium barnacles (t-test, $P=0.02$) but there was no effect of diet on growth for small and large barnacles (Fig. 5). Since diet was not a significant factor, those groups were pooled to find the average growth rates for small and large barnacles in mariculture, $1.05 \text{ mm} \cdot 8 \text{ wks.}^{-1}$ ($SD = 0.13$) and $1.34 \text{ mm} \cdot 8 \text{ wks}^{-1}$ ($SD = 0.46$), respectively.

Proportional growth was significantly different between size classes (ANOVA, $F = 9.43$, $df = 2$, $P = 2.59 \times 10^{-9}$). Small barnacles experienced higher percent growth relative to their starting size than medium and large barnacles (Fig. 6). The difference in proportional growth between diet treatments within the same size class was significant for large barnacles (t-test, $P=0.03$), but diet treatments had no significant influence on proportional growth for either small or medium barnacles.

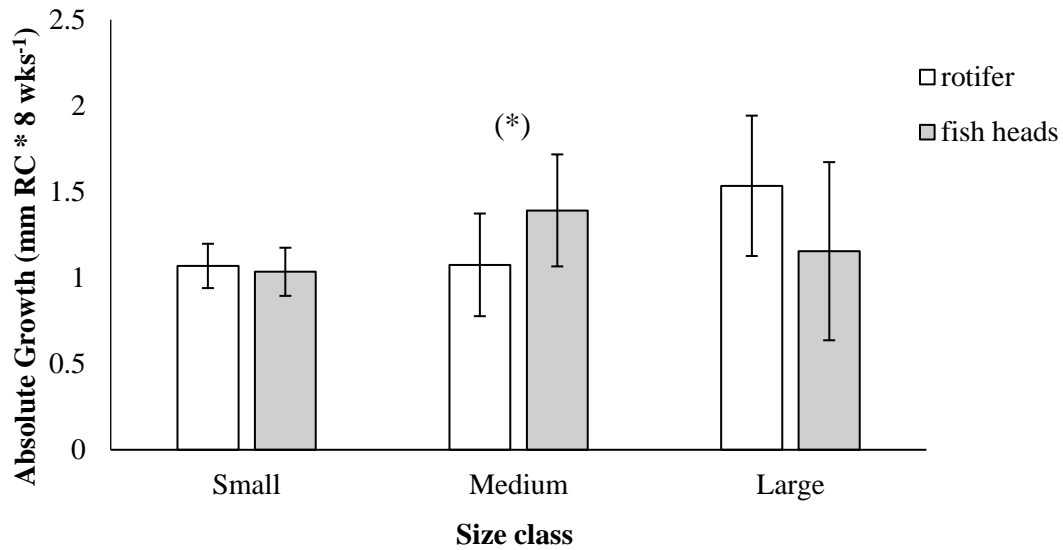


Figure 5. Absolute growth across three size classes (groups) and two diet treatments (shaded vs not) over the course of 8 weeks. The differences in growth rates between all three size classes were significant. (*) indicates where diet had a significant influence. Error bars represent standard deviation.

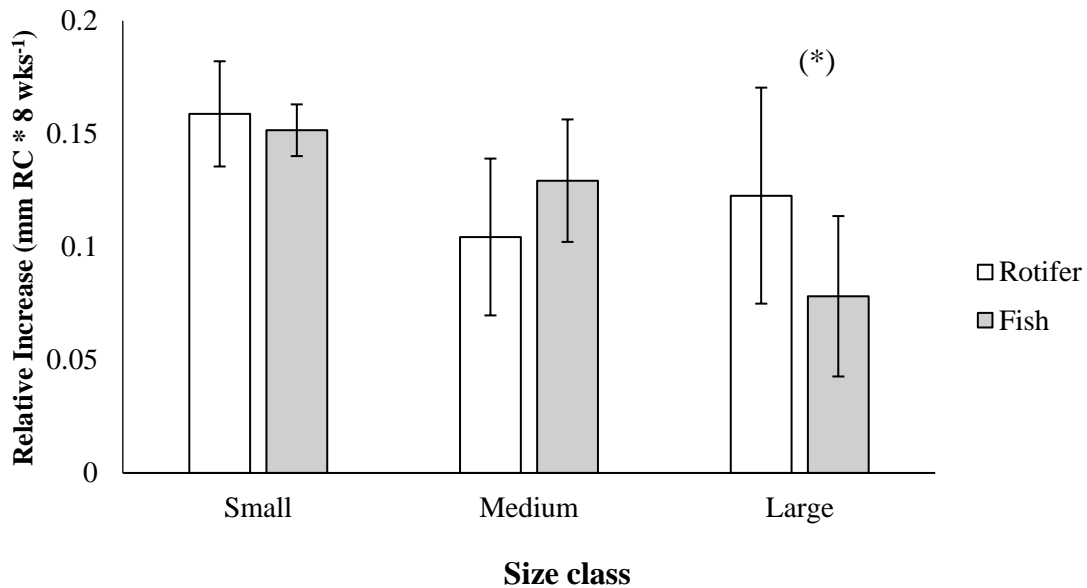


Figure 6. Proportional growth (percent gain) across three size classes and two diet treatments (shaded vs not) over the course of 8 weeks. Percent gain is measured in in mm RC ($RC_f - RC_i / RC_i$). The differences in growth rates between all three size classes were significant. (*) indicates where diet treatment had a significant impact on growth rate. Error bars represent standard deviation.

Experiment 1.2 “Semi-intensive” Settlement

In addition to growth, each barnacle was examined for cyprid settlements and new settlers were counted each week (Fig. 7). Cyprids were introduced to the mariculture system via the raw seawater pumped into the OIMB seawater system from the opening of Coos Bay to the Pacific Ocean. Over the course of nine weeks, small, medium and large barnacles accumulated a total of 343, 951, and 1269 new settlers, respectively. Settlement was not tracked for the first two weeks of the experiment and there were no visible settlers during weeks two to four of the experiment. Size class was a significant factor in settlement (ANOVA, $F = 30.03$, $df = 2$, $P = 2.27 \times 10^{-12}$).

An individual large barnacle on average accumulated 1.3x more settlers than individuals in the medium size class and 3.6x more settlers than individuals in the small size class (Fig. 8). There was no significant difference in the average number of recruits per barnacle between the different size classes on a weekly basis, however by week 9 the large barnacles were experiencing significantly higher per-capita recruitment than small barnacles (ANOVA, $F = 30.03$, $df = 2$, $P = 2.27 \times 10^{-12}$). There was a noticeable non-random distribution of cyprids on adult peduncles of the largest size class, with certain barnacles seeming to attract the majority of recruits while others in the same tank remained bare. The driving force behind this pattern was not rigorously investigated using statistical methods however it was a qualitative observation noted for potential future studies on *Pollicipes polymerus* settlement cues and substrate habitat.

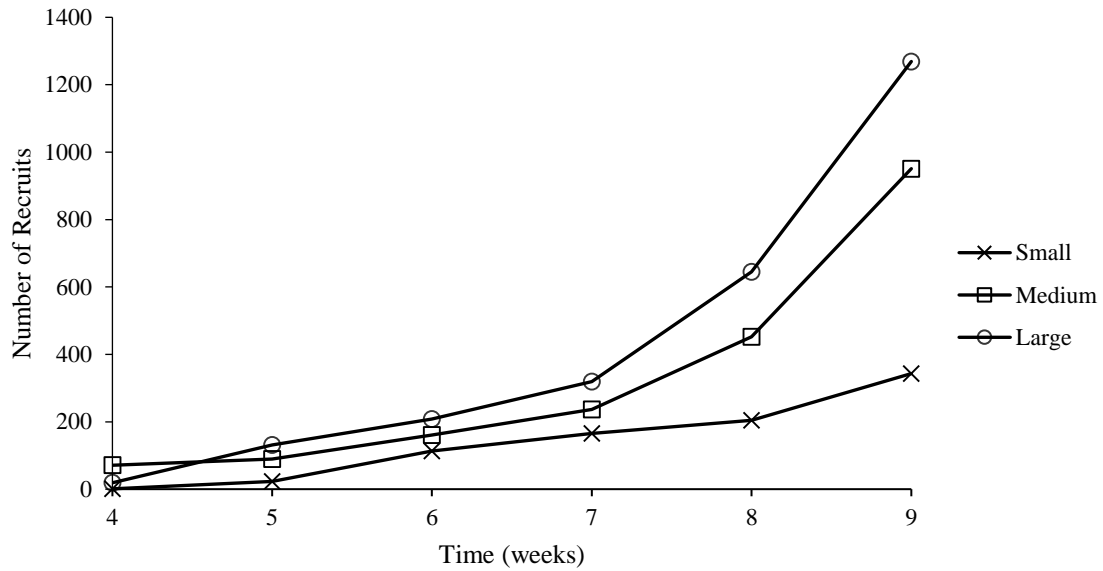


Figure 7. Total settlement on peduncles of three different size classes of barnacles over the last 5 weeks of a 9 week experiment, measured in number of settled cyprids on peduncles of adults in culture. There was no settlement observed during the first four weeks of the experiment.

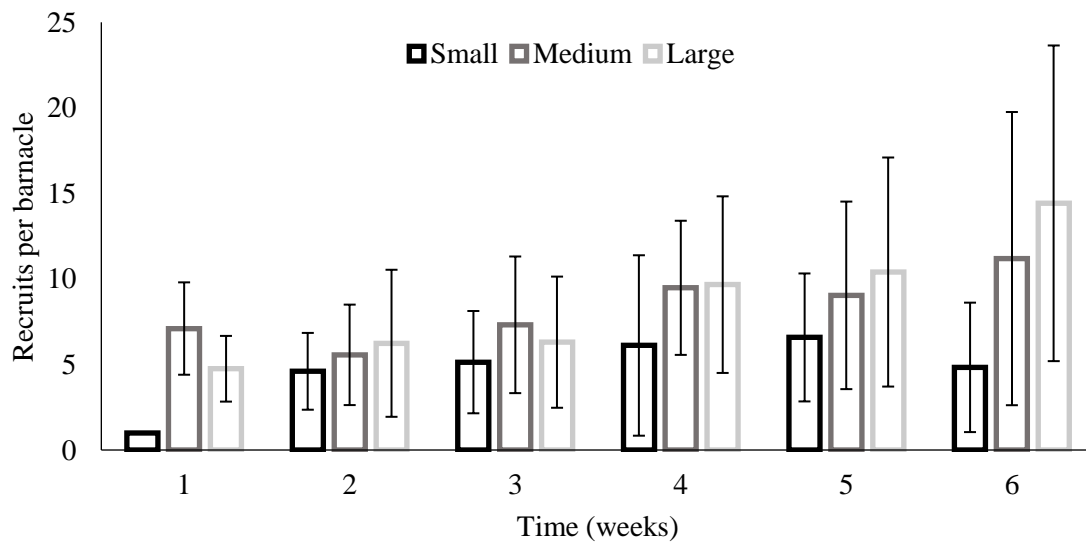


Figure 8. Per Capita recruitment on three different size classes of barnacles over the last 5 weeks of a 9 week experiment, measured as average number of settled cyprids per peduncles of adults in culture. Error bars represent standard deviation.

Not only were cyprids settling onto peduncles of mariculture individuals, but they were also growing; by week nine there were 63 instances of multiple recruitment classes living on the same peduncle (Table 1). Both attached cyprids and metamorphosed juveniles had to be present to qualify as harboring multiple recruitment classes. The maximum RC length achieved by a new recruit was 5.1 mm. Since there were no recruits observed for the first 4 weeks of the experiment, this represents a growth rate of 4.08 mm RC * month⁻¹, which is a magnitude higher than growth rates observed in adults in culture, which averaged between 0.3 – 0.8 mm RC * month⁻¹ (Fig. 4). This recruit was observed in a tank subjected to the rotifer diet treatment, however there was no correlation between diet treatment and average recruit growth rates.

Table 1. Total Recruitment, Per-capita recruitment, and number of individual adults with multiple size classes of recruits on one peduncle in the final week (Week 9) of Mariculture experiment, broken down by size class.

	Total Recruitment	Average Per-capita Recruitment		Individuals with Multiple Recruiting Classes
		Mean	SD	
Small (n = 71)	343	4.8	3.78	7
Medium (n = 85)	951	11.1	8.56	22
Large (n = 88)	1269	14.4	9.22	34

Experiment 2. "Extensive" Dock Experiment

Tanks were deployed off I dock in the Charleston Boat Basin for a period of two and a half months with no enhancement or manipulation other than a period of supplemental air that ended after two weeks due to the fouling of the aeration system. Barnacles ranged from 8.78 to 19.68 mm RC initial size. Of the 72 barnacles initially deployed, 21 survived to the final measurement (29% survivability). The innermost tanks (E and F) experienced 100% mortality by week 10. The tanks with the highest survival rates were C and D, each with 5 barnacles. There was no significant change in RC length over the course of the experiment (Fig. 9) indicating a growth rate of zero under the conditions that exist in the Inner Boat Basin (t-test, $P > 0.05$). All barnacle peduncles in this experiment were scraped clean of cyprids prior to deployment and there was no observed settlement over the course of the experiment.

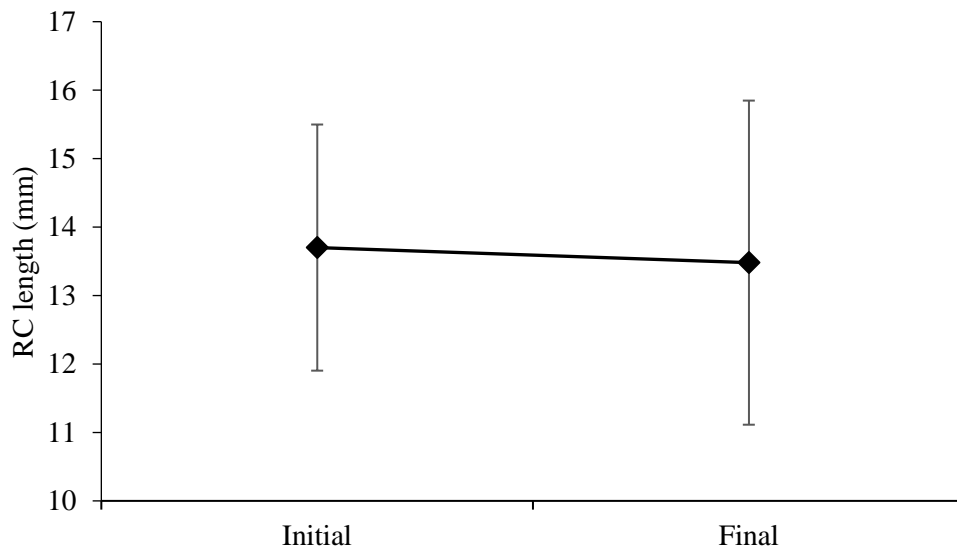


Figure 9. Growth of Barnacles deployed in Inner Boat Basin. There was no significant difference between starting size (mean = 13.41, SD = 2.2) and ending size (mean = 13.48, SD = 2.36), indicating a growth rate of zero.

Discussion

This study of mariculture of *Pollicipes polymerus* sought to establish successful culture conditions for gooseneck barnacles across three size classes fed one of two formulated feeds in a “semi-intensive” experimental culture trial that was paired with an “extensive” culture trial in the field. The context for this project, nested within a larger effort to explore the possibility of a sustainable gooseneck fishery on the Oregon coast, meant that each experiment was also designed with an eye to sustainability. Mariculture that strives to balance growth efficiency with sustainability is often characterized by practices that minimize waste, minimize feed inputs with detrimental externalities, and minimize impacts on natural environments.

Gooseneck barnacles, at first glance, are an ideal culture species, being sessile, benthic invertebrates that feed relatively low on the trophic chain. Other commercial species with a similar biological profile include oysters and mussels, both of which historically thrive in low impact “extensive” conditions that can be satisfied across a range of marine environments. However, gooseneck barnacles are kept from this category by two distinct features, one being feeding behavior and the other reproductive life history. Unlike mussels and oysters which are filter feeding broadcast spawners, gooseneck barnacles are passive feeders and, in the field, require heavy wave action in their natural environments to ensure food delivery and must remain in tightly packed aggregations to facilitate gamete exchange. Furthermore, seeding of gooseneck experimental cultures is inhibited by the highly selective cyprid larvae that settles exclusively on conspecifics. These life history traits complicate the possibility of scaling up to a commercial-sized culture system.

Experiment 1: “Semi-intensive” Diet Manipulation

In the “semi-intensive” culture experiment barnacles fed formulated diets in a lab setting achieved comparable growth to barnacles observed in the wild. Large barnacles in a semi-intensive culture system grew an average of $1.34 \text{ mm RC} * \text{ month}^{-1}$, which can be extrapolated to $16.1 \text{ mm RC} * \text{ year}^{-1}$. Average growth in natural environments for adult barnacles of a similar size class ranges from $15.7 \text{ mm RC} * \text{ year}^{-1}$ (Cruz et al. 2010) to $17 \text{ mm RC} * \text{ year}^{-1}$ (Lewis & Chia 1981b). Similar growth rates exist when barnacles reared from larvae and wild barnacles are compared (L. Hoffman 1989), (Molares et al. 1994). It bodes well for prospective aquaculture that barnacles harvested from the intertidal and replanted into a culture system are able to assimilate to culture conditions and grow successfully. While this system has been classified as “semi-intensive” for the purposes of comparison, it requires far less infrastructure than most mariculture systems of the same designation. Total system expenditures for all building materials did not exceed \$1,500, and feed cost, usually another big expense in aquaculture systems was low. Rotifer cultures are inexpensive and easy to set up and maintain. Fish waste, high in protein, locally abundant and free, was a proof-of-concept for sustainably sourcing cheap, high-quality feed.

Another important factor to consider when assessing the overall utility of this culture system is the capacity for each barnacle in culture to accumulate new, natural recruits. Within nine weeks, the tanks with 244 barnacles had accumulated a total of 2563 new recruits. Some barnacles had more than thirty cyprids and juveniles on one peduncle. Close to half of those had settled on the peduncles of large barnacles. Given the growth rates of the barnacles themselves, utilizing replanted barnacles as substrate for incoming

cyprids could generate far more barnacles in the long run than periodically harvesting cultured barnacles or replanting adults into the field to supplement natural harvest, both of which have been suggested as potential methods for mitigating the impacts of a harvest fishery. Other than barnacle size, there were no correlations between recruit count and any factor such as, diet, individual growth rate, or culture manifold that affected which barnacles were successful substrate and which were not.

Sources of Error and Unexplained Variability

Intertidal barnacles typically feed on small crustaceans and phytoplankton (Parada *et al.* 2012). Studies on physiology have identified the presence and interaction of multiple digestive enzymes, indicating a wide range of food items (Norton 1996). Norton (1996) also concluded that while they have the capacity to ingest and assimilate nutrients from a variety of food sources, specific enzyme activity indicates heavy preference towards carnivory. Given that preference, it appears that the high protein, nutritionally dense diets provided in these culture trials could be improved upon by more efficient infrastructure that provides food for more hours of the day to barnacles. In a recent study with a similar culture set up, Bingham *et al.* (2017) recorded growth rates between 1.1 and 1.6 mm RC * 8 wks⁻¹ for juveniles given no food subsidy and between 2.3 and 2.9 mm RC * 8wks⁻¹ for barnacles fed *Artemia* cysts. One potentially confounding variable is particle size, which confounds on two fronts. On one hand cirral morphology is specially adapted to an optimal particle size which changes as the barnacle grows (Marchinko & Palmer 2003) and two the size of the particle affects its buoyancy and therefore its retention time in the water column. The fish blend was ground to a size closely approximating rotifers, but there were both significantly larger and significantly smaller

sized particles with the batches of fishmeal. It is possible that six hours of feeding time was not adequate before new water flow was reinstated and food flushed out. This shorter feeding time could also potentially explain the lack of variation between diets. For the remaining 18 hours of the day when not feeding on specialized diets, these barnacles were exposed to the same population of plankton in the seawater system and were likely feeding on particles in the water. This may have overwhelmed any noticeable difference in growth rate based on diet. The only size class for which diet significantly affected differences in absolute growth was medium barnacles, and proportional growth in large barnacles. The growth rate of small barnacles did not vary with diet, however small barnacles did experience the highest average proportional growth. Given growth allometry, the varying “growth rates” measured are likely a consequence of shape and the discrepancy between linear growth measurements and volumetric growth. Since phytoplankton are the smallest and most abundant organisms in the pelagic environment and the ones most likely to be introduced to culture systems, it is possible that the smallest size class is utilizing this source of food in both the rotifer and fish blend diet treatment tanks, obscuring any statistically significant effect of the diets.

Experiment 2: Extensive Dock Experiment

The major challenge associated with this experiment was the delivery of air to the tanks, coupled with a location that was not well suited to gooseneck biology. On one hand, air flow was initially incorporated into the system via a ring of diffuser tubing at the bottom of each tank. The airflow calculations made before deployment indicated that the small aquarium pumps would provide adequate pressure to generate a curtain of bubbles. However expected air flow and observed air flow inside the tanks were vastly

different. A 1200 cc pump for pumping air underwater to a depth of 2m was insufficient. It was difficult to observe the bubblers in action at 2 m, as visibility is often far less than that in the Inner Boat Basin but judging from surface turbulence in the water immediately above each tank, there appeared to be a small steady stream of air. One solution for that is to decrease the depth of deployment of each tank, which was not possible for this experiment because the dock slip was in use by the owner and 2 m was the minimum depth to prevent damage to the tanks by boats moving in and out.

The low air pressure was made unacceptable in the experiment by the fouling of the diffusers further decreasing flow. The Charleston docks are ideal substrate for a robust and fast-growing fouling community, dominated by *Balanus spp*, *Mytilus californicus* and *Botrylloides violaceus*. *B. violaceus* grew with zest on all submerged surfaces, including the diffuser. Despite bi-weekly tank maintenance, the encrustation by combinations of tunicate and barnacles overwhelmed the air delivery system and the goosenecks were left to depend on ambient flow. The tidal induced flow in the Inner Boat Basin is not enough for growing gooseneck barnacles, although barnacles in these experiments were exposed to a more typical diet of mixed diatoms, crustaceans and the vast larval supply from the fouling community. Unfortunately, any benefit afforded by this food source was outweighed by the lack of water movement. Without water movement to induce feeding behavior, barnacles were likely only feeding during short periods of time immediately after measurement days when they were plunged back into the water column and their tanks cleared of obstructions to flow. There have been some noted instances of adult barnacles extending their cirri in the absence of turbulence in a slow, rhythmic beating pattern but it is thought that this behavior serves a respiratory

function rather than a feeding one (Marchinko & Palmer 2003). Without being able to feed, many of my experimental barnacles experienced mortality.

The incidence of mortality in this experiment was heavily tied to water flow, with barnacles in tanks E and F on the inside corner of the boat slip (where flow is consistently near zero) experiencing 100% mortality by the end of the experiment. The barnacles in tanks A-D experienced some mortality, ranging from 33% to 75%. It is unclear what percentage of this could have been due to predation, although the tanks were relatively enclosed and suspended from ropes. While it is not unheard of for *Pisaster ochraceus* to crawl down a rope, it is unlikely in this system (pers. comm, Z. Knorek). The hanging dock experiment could be improved with stronger air flow, tanks closer to the surface, and higher flows up to that of the natural habitat.

Conclusion and Future Directions

This system of vertical tanks housing multiple barnacles in a controlled flow-through water exchange, could not be scaled up to meet the demands of a commercial fishery. The infrastructure does not lend itself to expansion, nor does the feed production system. The tanks were uniquely adapted and built to cater to specific biology of the gooseneck barnacles living inside them, which also means that they would not be ideal for a more complex farming system such as one that involves polyculture or crop rotation. However, individual components are useful for moving forward with gooseneck culture, specifically using air to trigger feeding, utilizing recycled fish waste to create a formulated feed, and attracting cyprid settlers with in-planted adults. The up-cycled fish represents a significant step towards sustainable aquaculture and aligns with the literature

on the use of industrial fishing by-products as a way to not only increase the value of the wild caught fish but also increase the efficiency of the system in which it is utilized, in this case the gooseneck barnacle farm. The processing of recycled fish by-product at this scale required minimal equipment and manual labor, however it would likely require more extensive supporting production systems if scaled to a commercial size.

Moving forward, the most pressing logistic is scaling up air flow but maintaining the ability to control culture conditions, since initiating feeding behavior is clearly the baseline priority. The next step might be to test a functioning recirculating system with an automated feeder that deposits food on a schedule, to ensure maximum feeding time. It would also be useful to quantify whether settlement patterns in culture reveal a preference for adult barnacles that already have cyprid recruits. The high incidence of multiple recruiting classes on a single peduncle indicates that certain barnacles might become “favorites” which is useful information for both the application of aquaculture as a potential method to increase *in vitro* biomass or as a potential subsidy to the intertidal in a replanting scheme.

Chapter II concludes that growth is possible in a culture system. However, merely achieving growth is not enough to label an aquaculture operation successful: in this day of limited resources and rampant environmental degradation at the hands of humanity, such systems must also be sustainable. Sustainability in aquaculture can be defined many ways. Chapter III will investigate some of the standard metrics for evaluating sustainability, apply them to gooseneck barnacle aquaculture, and compare to other marine species within an efficiency matrix.

CHAPTER III

BIOMETRICS AND FEED EFFICIENCY

Introduction

As aquaculture gains ground in the conversation about global food production and food security, industry challenges arise from the higher demand for fishmeal and fish oil to support increased farming efforts. Aquaculture has typically relied on natural “forage” fisheries to provide fishmeal and fish oil for feeds (Raghukumar 2008). Forage fish are fish that are smaller, bonier, or less nutritious than is ideal for human consumption and as such are often viewed as less threatened by harvest pressure than other desirable fish species such as salmon or tuna (Pikitch et al. 2014). However, aquaculture is the fastest growing food-producing sector in the world and as it continues to expand there is increasing concern about the additional strain on global forage fish stocks and the detrimental ecological effects of industrial fishing for forage fish (Tacon & Metian 2008).

As the gap between supply and demand continues to increase, research that examines alternatives to fishmeal and fish oil has gained popularity and relevance (Table 2). Improvement in alternatives such as plant- and microbe-based lipids, rendered terrestrial products, and seafood bycatch, are expected to lead to more efficient use of marine resources in the long run (Miller et al. 2008). However, “efficiency” can be calculated numerous ways, making it difficult to standardize and compare across systems. This buzzword, ubiquitous in the literature, consolidates various economic and biological factors into one concept at the expense of resolution and specificity and can refer to a wide range of industrial conditions.

Table 2. Alternate Sources of macronutrients in Aquaculture

Feed Type	Source	Pros	Cons	Reference
Plant-based proteins	Reconstituted barley, canola, corn, soybeans	Cheap. Renewable. Easy to scale up production.	Existing land-use issues. Low fiber and starch content, high indigestibles.	(Gatlin et al. 2007) (Samocha 2004)
Plant based lipids	oils from canola, sunflower, olive, palm plants.	Cheaper than fish oil. Renewable. Easy to scale up production.	No/Low concentration of (ideal) LC-PUFA omega-3s and omega 6's	(Trushenski 2009) (Torstensen et al. 2005) (Bell et al. 2004)
Single cell proteins/lipids	Protists (thraustochytrid), diatoms, bacteria	Renewable. Nutrient dense. ideal fatty acid profiles for many marine larvae	Expensive to scale up for a commercial operation	(Raghukumar 2008) (Carter et al. 2003) (Barclay & Zeller 1996)
Animal proteins/lipids	Rendered blood, bone, feather organic matter from terrestrial env.	Complete amino acid profile. Cheap way to upcycle agricultural waste	High in saturated fats, resulting in low digestibility	(Hardy 2009) (Tacon et al. 2006)
Genetically Modified Organisms from Terrestrial Environments	GMO grains for increased protein yield, oil-producing plants and micro-organisms	Modified to produce long-chain omega 3s or higher concentrations of protein	Negative consumer perception	(Robert 2006) (Abbadi et al. 2001)
Seafood by-products	Processing-plant waste, discards from docks	Ideal nutrient profile and amino acid composition. Cheap way to upcycle industrial waste	Incentivizes potentially detrimental bycatch Degenerative Prions	(Huntington 2007) (Forster et al. 2005) (Allan 2004)

For instance, plant based proteins (e.g., barley, corn, and soybean derivatives) are considered “efficient” because they are cheap, but these feeds contain a large proportion of indigestible organic matter and thus increase the amount of excrement and waste in fish farms (Samocha 2004). In contrast, protein and oil derived from single-celled organisms such as protists in the thraustochytrid group, are also considered “efficient” because they are easily digested and more nutrient-dense per gram than other marine-derived food sources, but scaling micro-organism production up to a commercial level is labor intensive and expensive (Carter et al. 2003). Rendered terrestrial protein, another option that has recently gained traction, is less expensive and contains a more complete amino acid profile, but is typically high in saturated fats, which makes it more difficult for marine species to digest (Naylor et al. 2009). Even among the fish-meal and fish-oil based diets, efficiency within a single group can vary according to the digestion, absorption, accumulation, and metabolism of different species in response to environmental variable such as temperature, stock density, or water chemistry (Monroig et al. 2013, Liu et al. 2018).

Despite this ambiguity, there are methods for analyzing the efficiencies of different systems in a way that allows comparisons to be made. One such standardized metric is Feed Conversion Ratio (FCR hereafter), which is calculated as $(\text{Net Feed Input}) / (\text{Net Production})$. FCR allows for multi-scale comparisons and has been used to analyze between systems and across taxa or can be used to compare between two experimental feeds in the same system. The lower the FCR value, the more efficient a system is. FCR values track closely with trophic positioning; low-level primary consumers typically convert food more efficiently in culture condition and have low FCR scores, while top

carnivores require more food to produce the same amount of tissue and have high FCR values (Gephart et al. 2016).

As research attention increasingly focuses on solving sustainability issues in aquaculture, the FCR efficiency gap between feeds low and high on the food chain, and more widely between marine and terrestrial industrial food production systems continues to widen, with studies showing that marine hatcheries are more efficient across the board than land-based husbandry operations (Fry et al. 2018). The least sustainable marine farms that grow large carnivorous fish such as salmonids that rely on energy dense nutrients and feeds made with high quality ingredients are still far more sustainable than terrestrial species of a similar nutritional and economic value such as cows or pigs (Liu et al. 2016). Marine farming operations benefit from the natural ubiquity of essential fatty acids (particularly n-3 long chain poly-unsaturated fatty acids (PUFA) such as EPA and DHA) in marine environments, originating with microscopic marine algae and manifest in marine fish and invertebrate groups higher in the food chain (Monroig et al. 2013). These PUFAs are modified as they move up the food chain making seafood the most important source of essential fatty acids in the human diet (Tur et al. 2012).

This advantage is evident at the top of the food chain but begins at the bottom. Many marine plankton often used as live feeds in aquaculture display efficient absorption and biosynthesis of desirable nutrients and fatty acids (Brett et al. 2009). For instance, copepods are able to synthesize desirable long-chain PUFAs from a low-quality diet of detritus (Drillet et al. 2006), and rotifers are used to enhance larval growth because they uptake n-3 highly unsaturated fatty acids from enriched diets and those fatty acids are

transported up the food chain (Hamre 2016a). Both live feeds are commonly utilized to increase essential lipids in the diets of larval fish and crustaceans.

It is important to design feeding regimes in aquaculture systems that incorporate both appropriate fatty acid profiles and macronutrient requirements. The typical consideration of diet composition when designing formulated feeds for commercial species centers around proteins, lipids, and carbohydrates and ratios thereof. Of the three, protein is often the most important factor in determining feed composition since it is the macronutrient that contributes directly to tissue building and growth (Craig & Helfrich 2009). Protein content is used to assess net production between systems, since it is also the most important nutrient for human diets and the one that is prioritized in conversations about food security and availability. The provision of cheap protein is therefore desirable in aquaculture and is a major factor in the overall value and sustainability of gooseneck barnacle aquaculture.

Gooseneck barnacle aquaculture is dependent on the appropriate input of protein, as all harvest operations are. However, a lower metabolism means that diet can cater to economic efficiencies without a significant loss in biological efficiency. The formulated feeds for gooseneck barnacles utilized in this study are desirable in both their biology and economy. Feed Conversion Ratios, protein content, protein retention, body condition, and proportion edible are vital metrics in aquaculture and knowledge of these for other species have existed for years and are being continuously updated as systems become more efficient and feed technology continues to become more sophisticated. These metrics provide a baseline upon which to build a more involved and rigorous aquaculture system. For gooseneck barnacles, which are of economic value on every coast they

inhabit across their global range, this bioenergetics matrix provides a compelling argument for using aquaculture to potentially supplement natural harvest.

Investigating Gooseneck Efficiencies

In this study, I aim to investigate the FCR value, protein content, protein retention, and edible portion for *Pollicipes polymerus* and the diets utilized in this experiment, where appropriate. Barnacles were fed either fish blend or rotifers and every feed input was weighed and recorded while barnacle growth was tracked via mass measurements over the course of eight weeks. Overall waste from the system was also recorded and protein assays were performed to assess protein content of both peduncle meat and feed sources. These values were then used to calculate protein retention, which is the measure of how efficient an intermediary organism is at making protein available to the final consumer in a food chain or in this case, industrial system. “Edible portion” describes the percent of total biomass that is edible for humans. These measures of biological efficiency exist for other marine species of commercial interest but have never been reported for Gooseneck Barnacles. One reason to study barnacles through the lens of efficiency in aquaculture is to prepare information for future aquatic farmers who might be interested in introducing *Pollicipes* into a culture set up. The metrics investigated in this study are universally used to compare different farming enterprises and species against one another and provide essential nutritional information to the final consumer.

Materials and Methods

Collection

Pollicipes polymerus were collected from South Cove, Cape Arago, Oregon (43° 18' 10.242" N, 124° 23' 58.5816" W) on 03 May 2018. Care was taken to only scrape small barnacles (approximately 10 mm RC and less) from the rocks for use in this experiment. Sixty barnacles were chosen from the sample and each was weighed prior to being installed in the tank system. Each barnacle peduncle was scraped of attached juveniles after being brought in from the field. Additional barnacles were collected for length-weight relationship metrics on 07 June 2018 and included barnacles from all size classes.

System Construction

In order for FCR to be measured properly, the experimental culture trials were undertaken within a closed tank system. Within an array of twenty individual 250 mL jars, ten were designated to receive fish blend treatment and ten were designated to receive rotifer treatment. Each set of ten was connected to a ten-point air manifold with 6.5 cm tube sections and pumped with continuous air from the campus air supply. Air was introduced into each tank via coil of diffuser tubing at the bottom. Barnacles were attached to clothespins after they were attached to Velcro squares and each jar housed three barnacle-laden clothespins, for a total of 30 barnacles per diet treatment and 60 barnacles total. The system was designed this way to make weekly weigh-ins more feasible, as each barnacle was easy to separate from its tank, weigh, and then reattach immediately afterwards. Each barnacle remained attached to the same Velcro square for the duration of the experiment. The barnacles were oriented downward so that their

rostral carinal openings were positioned to induce feeding behavior and orient towards the food that remained suspended with the bubbling action. The goosenecks on their pieces of Velcro could be easily removed from their clothespins during tank flushing and once every week for measuring biomass gain. During the weigh-in process, each barnacle and the attached Velcro square were weighed using a high precision scale. Specimen were allowed to dry before each weigh in to prevent water retention confounding growth measurements. While air was pumped continuously, there was no water flow, other than turbulence caused by bubbles. Each tank was emptied and refilled every day with filtered seawater that contained a specific food concentration.

Diet Treatments and Waste Collection

Live Rotifers (*Brachionus plicatilis*) were administered at a density of 9600 ind/L. Fish blend was created according to the procedures outlined in Chapter I and was administered at a concentration of 881 mg/L. To ensure that the two feeds were as close as possible in terms of volume, concentrations of fish blend were calculated based on the maximum ingestion rates of 81 rotifers/hour reported by Norton (1996) for adult *Pollicipes polymerus* (RC length >15mm). Adult ingestion rates were used as a deliberate over-estimation for the small barnacles in this experiment to ensure that barnacles were fed to satiation. Fish blend concentration was then matched as closely as possible to rotifer concentration based on reported rotifer mass in the literature at 0.12-0.36 μg per individual (Hoff & Snell 1987). Feeds were premixed into 2 L of filtered seawater and the food-laden water was used to refill the tanks every day after emptying and cleaning. After splitting across ten tanks, final rotifer density per tanks was 1920 rotifers tank⁻¹, or 640 rotifers barnacle⁻¹ day⁻¹ which matched the final concentration of fish blend

particles per tank. The feeds were not exactly mirrored in biomass (more fish blend was added by mass each feeding time) because fish blend concentrations were corrected upwards in order to account for particle interaction with the foaming meniscus of each tank, which removed approximately 30% of particles from circulation.

Waste was collected by draining the contents of an individual tank through a standard coffee filter, with mesh size approximated at 10-20 microns. The filters were allowed to stand and drain for 12 hours, then were dried to eliminate water weight and the mass of waste measured. All filters were weighed prior to use. There was no way to rinse filters without losing waste, so a small percentage of the mass arises from the salt content of the seawater. For every liter of seawater there are 35 g of salt, which must be accounted for. 250 mL of filtered salt water was strained through a coffee filter to test residues, and subsequently 0.18 g was subtracted from each waste measurement as a baseline salt accumulation on the filter.

Protein Analysis

Peduncle muscle from wild barnacles and fish blend feed were analyzed for protein concentration using the Bradford Protein Assay, one of a suite of widely utilized proximate composition analysis procedure (Bradford 1976). Protein assays were performed on 26 April 2018 using a Genesys 20 Visual Spectrophotometer. The standard curve was calculated using Bovine Serum Albumin at stepwise dilutions of 2.5-100 ug/ μ L. Rotifer protein analyses were attempted but tissues proved to be in too low a quantity for the procedure to be effective. In the Bradford Protein Assay, samples must be freeze-dried prior to analyzing protein content and the desiccation process reduced rotifer tissues to far below a useable yield. The literature reports protein levels in rotifers to be

relatively stable at 40%, although protein is variable based on the genetic strain of rotifer (Hamre 2016b).

Edible Portion Analysis

Barnacles of commercial size (>20 mm RC) were collected from South Cove and mass was measured while barnacles were alive and whole. Barnacles were then frozen in order to expedite processing and the capitulum was separated from the peduncle with a razor blade. The peduncle was skinned (the outer chitinous sheath separated from inner muscle) and the final mass of the peduncle muscle was measured. Only barnacles that were of an appropriate size and proportion were chosen for this measurement.

Appropriate here refers to commercial-sized barnacles of Rostral Carinal lengths greater than 20mm that looked to have a long and muscular peduncle prior to being scraped off the rocks. Only the largest barnacles were collected because small size classes are not consumed by humans

Data Analysis

All data were analyzed in R (R Core Team 2013). Growth data was untransformed, as mass is a relatively stable measurement and there is less precedent in the literature for transforming mass data than other types of growth metrics (Ranganathan & Borges 2011). Proportional growth was calculated using Equation 1. After this calculation a log transformation was used and all statistical tests were performed on log transformed data.

$$Eq. 1: \frac{Final\ Mass - Initial\ Mass}{Initial\ mass}$$

Growth and the log of proportional growth were compared across diet treatments using a one-way ANOVA and standard deviations (SD) were calculated based on a normal distribution. All the barnacles were of the same size class and the experiment was performed in a closed system with no other confounding variable. Diet was the only source of variance tested using statistical methods for the raw data. Other data are the result of calculations using the following set of equations utilizing untransformed data. Feed conversion ratios (FCR) were calculated using Equation 2. The reported mass of 2.34 µg dry weight/individual from the literature was used to calculate the total feed input of rotifer feed by mass (Hoff & Snell 1987). Net production refers to the sum total biomass of all organisms per diet treatment at the end of the experiment.

$$Eq. 2: \frac{Total\ Feed\ input\ (g)}{Net\ produciton\ (g)}$$

FCR is a straight measure of the productivity of the system, however, the sustainability of a system depends on the balance of production and waste. The sustainability score for each feed was calculated using Equation 3. “Waste” is the total accumulated waste from each diet treatment, it does not distinguish between uneaten food and excrement.

$$Eq. 3: \frac{Total\ Feed\ input\ (g)}{Net\ produciton\ (g) - Waste\ (g)}$$

Another important metric included in the final table of values is protein retention, which refers to the amount of protein that is available to the final human consumer based on feed inputs. Higher protein retention is desirable and indicates a high overall level of efficiency. This calculation does not take into account the various chemical reactions

occurring inside the organism that might be transforming uptake protein into different forms to use for different metabolic functions. It is purely based on overall protein concentration and mass. This is calculated using Equation 4.

$$Eq. 4: \frac{\text{final protein for consumption}}{\text{protein in feed}} = \frac{\frac{g \text{ edible flesh}}{g \text{ whole animal}} \times \frac{g \text{ protein}}{100 g \text{ edible flesh}}}{FCR \times \frac{g \text{ protein}}{100 g \text{ feed}}}$$

Comparing Exp. 1 (Open Tank) and Exp. 2 (FCR) Growth

Experiment 1 measured growth as the change in mm Rostral Carinal length over the course of a feeding experiment utilizing rotifers and a formulated fish blend feed, as outlined in Chapter I. Experiment 1 (E1) was open to raw seawater and subject to fluctuations in water flow to a degree that Experiment 2 (E2) was not. E2 was a closed system using filtered seawater with a much higher feed input per barnacle. Growth was measured as the change in (mg) of mass. These two systems were tested against the same diets (live rotifers and emulsified fish blend) and represent two versions of a gooseneck barnacle aquaculture prototype. The smaller, closed system of E2 is a production method that would be appropriate for the younger barnacles and the larger scale system of E1 represents a version that caters more broadly to the range of size classes that would be found in a natural clump in the field, comprising the large size class of commercial value. Integrating these two experiments for comparison provides a more complete understanding of the utility and applicability of both feeds and culture infrastructure for growing goosenecks.

Before analyzing comparisons between experiments, the growth data (in mm Rostral Carinal length) from E1 was log transformed and the growth data from E2 (in mg

biomass) was tested for normality (Shapiro-Wilkes Test, $P > 0.05$). Experiment 1 used Rostral Carinal length as a proxy for overall growth, because the mode of attachment to PVC rods was not conducive to measuring mass for a time series. Experiment 2 directly measured mass and therefore did not use the proxy of RC length to estimate growth over time. Length-Weight Relationship for barnacles in Experiment 2 was determined in order to provide a regression coefficient for converting RC length into mass for comparison.

The Rostral Carinal lengths of all FCR barnacles were measured in the last week in order to build a Length-weight relationship (LWR) curve (Fig. 10). The given regression equation of $y = 5.8487x + 6.7435$, where y is RC length and x is mass, reflects the proportional relationship for anatomies of juvenile barnacles. However, *Pollicipes* does not exhibit linear growth in the wild (Barnes 1996) and so to compare the juvenile barnacles of the FCR system experiments against the range of size classes in the open tank system experiment, barnacles collected from the intertidal zone representing a range of sizes were measured and another LWR curve calculated (Fig. 11). This polynomial curve is more representative of a lifetime of growth for *Pollicipes polymerus*, where growth is logarithmic over time ($y = -0.4668x^2 + 5.2747x + 7.7652$). This equation was used to transform the RC length data of small barnacles from E1 into mass, to compare to the mass of barnacles in E2.

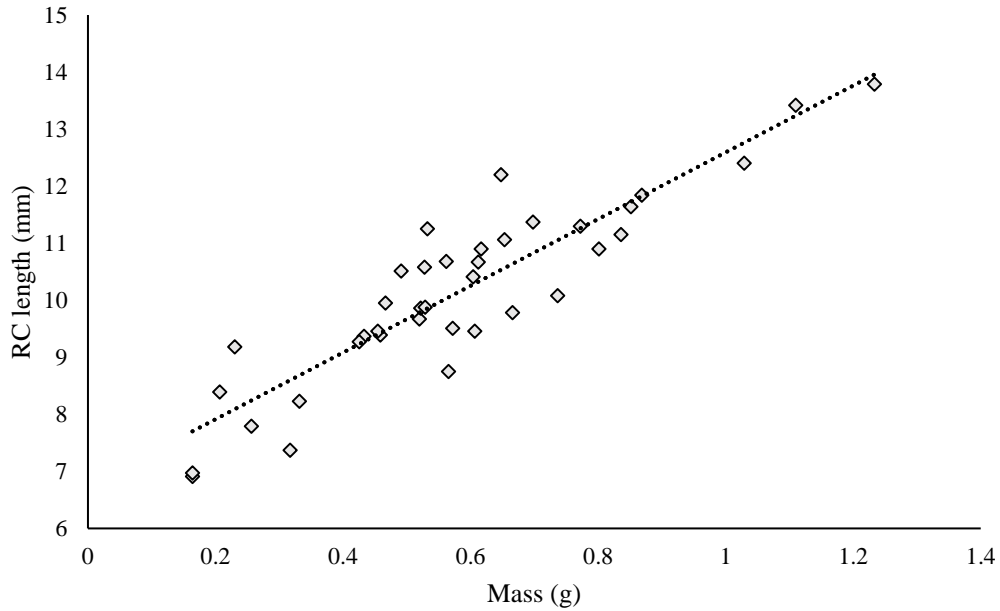


Figure 10. Length Weight relationship for barnacles in FCR experiment, measured at the end of a month of growth in lab conditions. The regression line is linear and is represented by the equation $y = 5.8487x + 6.7435$, $R^2 = 0.815$ ($n = 39$).

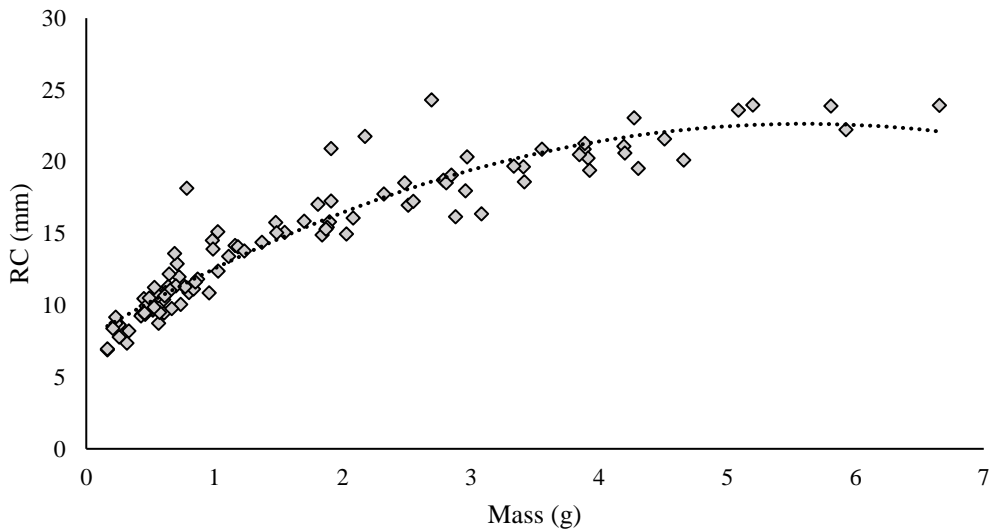


Figure 11. Length Weight Relationship for barnacles of all three size classes, including barnacles from the FCR experiment and barnacles collected from the wild. The regression line is polynomial and is represented by the equation $y = -0.4668x^2 + 5.2747x + 7.7652$, $R^2 = 0.8967$ ($n = 104$).

Body Condition Index

The length-weight relationship is a body index commonly utilized in aquaculture and is useful for comparing across systems that utilized different growth metrics (see previous section), but also provides information about body condition. Body Condition Indices are used in aquaculture as a way to test the health of an animal without having to conduct expensive and time consuming physiology tests (Labocha et al. 2014). Body Condition for most farmed marine invertebrates is calculated by measuring weight coupled with another size factor such as shell length in *Crassostrea* spp. (Pieterse et al. 2012) and *Mytilus* spp. (Mubiana et al. 2006). No body condition index exists for *Pollicipes polymerus*. If a barnacle falls outside the normal variation for the RC : Mass ratio, it could be an indication of malnourishment or some other general malaise. This metric is inexact by design, it is meant to provide a snapshot of the health of the animal, which can then be used to assess further action in an aquaculture setting. The condition index is calculated using the same regression technique as LWR but is represented as a single number, RC : Mass.

Results

Growth

Barnacle growth was measured once per week over the course of one month during which time barnacles were receiving the same amount of food every two days. Barnacles fed fish blend grew faster than barnacles fed rotifers for both growth metrics: total growth over time (ANOVA, $F = 5.73$, $df = 1$, $P < 0.05$, Fig. 12) and percentage increase in biomass over time (ANOVA, $F = 5.76$, $df = 1$, $P < 0.05$). Barnacles on the fish

blend diet gained 297.3 mg (+/- 153.7 mg SD), which represents an average 76% increase in biomass (SD 0.46%) (Figure 2.4) and barnacles fed live rotifers on average gained 199.9 mg (+/- 138.3 mg SD), which represented an average 50.8% increase in biomass (SD: 0.24%) over a period of one month (Figure 13).

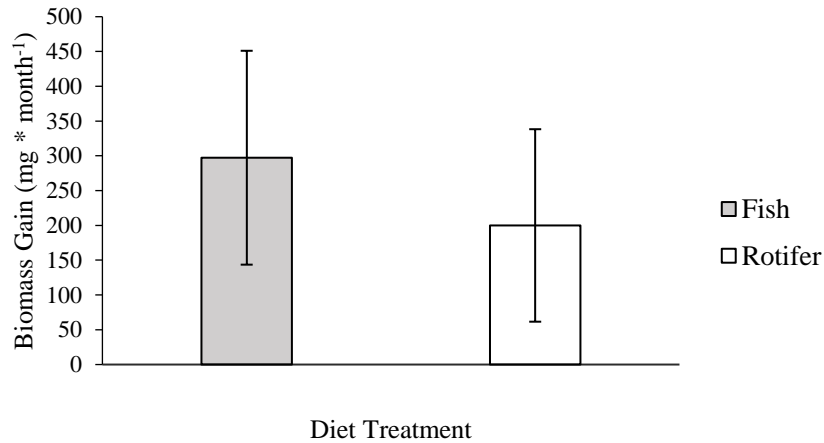


Figure 12. Difference in growth, measured as (final biomass – initial biomass), between barnacles fed fish blend diet (grey shaded) and live rotifer diet (unshaded) over a period of one month. Differences in growth based on diet were significant ($P < 0.05$). Error Bars represent standard deviation.

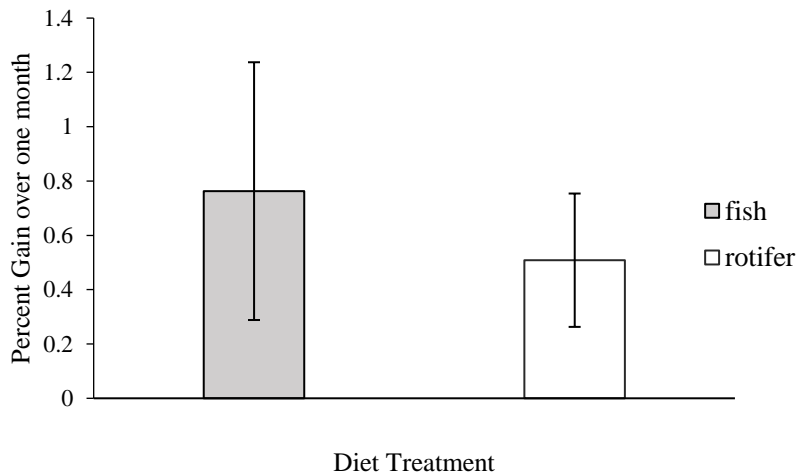


Figure 13. Difference in percent gain (final biomass – initial biomass / initial biomass), between barnacles fed fish blend diet (grey shaded) and live rotifer diet (unshaded) over a period of one month. Differences in proportional growth based on diet were significant ($P < 0.05$). Error Bars represent standard deviation.

Feed Conversion Ratio

Feed, waste, and barnacle mass were all measured on a bi-weekly basis. FCR values, the ratio of feed input to net output, measured in mass of organisms, exist for each diet type and represent total accumulated biomass over the course of one month. The measured FCR for barnacles consuming fish blend was 1 : 1.69 grams fish blend for every gram of barnacle produced. This is comparable to the feeding efficiency of chickens fed enhanced grains (Fry et al. 2018), The FCR value for barnacles consuming live rotifers was 1 : 0.09 grams of rotifers for every gram of barnacle produced. This FCR value was calculated according to Eq. 2, using the mass of rotifers as reported in the literature at $2.34\mu\text{g rotifer}^{-1}$ dry weight. Thus, rotifer mass is only an approximation and was not directly measured for each feeding in this experiment. Even accounting for discrepancies in mass per rotifer in the literature, such a low FCR value seems to defy the law of conservation of mass.

However, Feed Conversion Ratios of less than 1 : 1 are possible with commercial diets, as the feed is a "dry" diet, and a high percentage of weight gained by the organism is water trapped in the tissues and cells. Since rotifer mass was not directly measured in this experiment but rather was searched in the literature and reported in dry weight, this value may stand. However, the parallel FCR value calculated using actual rotifer density is 4.12×10^4 rotifers per gram of barnacle, which is more useful given that standard procedures for administering rotifer feeds in a hatchery environment rely on culture density rather than mass, however less widely applicable for comparing to other farmed species (Table 3). The Feed Efficiency score is a sub-set of FCR and calculated using Equation 3. This score incorporates the additional factor of waste production and

mortality (in this case 0) and is used to represent the efficiency of food use. It is the inverse of the FCR value, and therefore higher numbers represent more sustainable systems.

Table 3 Input values contributing to Feed Conversion Ratio calculations for barnacles fed fish blend and rotifer diets. Rotifer FCR values are represented by both mass (g) and count (rotifer density) to better describe the ratio.

Comparison of FCR values across Diet Treatments			
	Fish Blend	Rotifers	
		By mass	By count
Food input	14.1 (g)	0.045 (g)	211200
Biomass gain (g)	8.32	5.198	5.198
Total waste (g)	2.64	2.11	---
Feed Efficiency (1/FCR)	0.59	11.1	---
FCR	1:1.69	1:0.095	4.12x10⁴ rot*gram⁻¹

Comparing Growth in Exp. 1 (Open Tank) and Exp. 2 (FCR)

Percent gain between experiment as well as the difference between diet treatments within the same experiment were higher in the FCR system than in the Open tank system (Table 4). Percent gain is used for analyzing data sets where the starting condition, in this case initial size, is variable. There was higher growth across all diet treatment in the FCR system (ANOVA, $F = 170.2$, $df = 1$, $P < 0.05$). The regression equation for barnacles of all sizes (Fig 2.4) was used to estimate the mass of barnacles in Experiment 1 ($y = -0.4668x^2 + 5.2747x + 7.7652$). Barnacles mass, measured in E2 and calculated via LWR coefficient for E1, was used to compare proportional growth between the two systems. Small barnacles growing in a closed system fed exclusively a formulated diet in filtered

seawater grew 35-60% more than barnacles of a similar size class in a flow-through system with potential interaction with wild plankton (Table 4).

Table 4. Percent Gain comparison between E1 (Open tank) and E2 (FCR) based on LWR regression. Small size class used for analysis.

	Experiment 1 (Open Tank)		Experiment 2 (FCR)	
	Mean	± SD	Mean	± SD
Fish Blend	0.150	0.063	0.763	0.483
Rotifers	0.155	0.048	0.508	0.250

The body condition index for barnacles is 12.56 (n = 90). This index is a comparison of RC length to Mass, two common metrics of growth. Body condition is useful for taking a quick snapshot of the overall fitness of a clump of barnacles, or as a baseline index of fitness for assessing responses to stress.

Protein Assays and Retention

Spectrometry and biochemistry revealed the percent protein of total mass in both fish meal feed and barnacle peduncle muscle tissue. Feed protein content ranged from 49.7-57.3 g per 100g feed for fish viscera (mean = 53.50, SD = 3.79 n = 6). Rotifers fed *Nannochloropsis* are reported in the literature to contain an average of 40 g protein per 100 g (Hoff & Snell 1987). Rotifers were not included in this protein assay because cultures were not robust enough to sustain the volume of harvest required to accumulate the required biomass for the Bradford Protein Assay. Protein content of adult peduncle ranged from 38.1-56.5 g per 100 g tissue (mean = 47.38, SD = 9.26, n = 20).

Protein retention was calculated using Equation 4 for both fish blend and rotifer diets. Protein retention for barnacles fed fish blend ranged from 76.6% to 98.6% and ranged from 69.8% to 80.4% for barnacles grown on rotifers. This refers to the

percentage of original protein introduced into the system that is still available to the final (human) consumer.

Edible Portion

Large, commercial-sized barnacles were used for measuring the edible portion of barnacles because smaller size classes are not eaten by humans and therefore the edible portion of a smaller barnacle is irrelevant for comparing to other species of commercial value. Total mass of barnacles ranged from 1.59 g to 12.67 g (mean = 5.17, SD = 2.4). The average ratio of Edible Portion : Total Biomass was 0.25 (SD = 0.03). This is lower than most terrestrial sources of protein but comparable to other marine species that are consumed for their meat (Table 2.7).

Discussion

Aquaculture is essential to the future of global food production and security, and the success of aquaculture depends on its ability to remain sustainable. Sustainability has been defined by Tlustý & Thorsen (2017) as “behavior that drives economic, environmental and ethical progress towards ensuring that seafood availability meets the needs of the present without compromising the ability of future generations to meet their own needs”. One common indication of sustainability in fin-fish aquaculture is a low net-use of carbon and reduced impacts on natural systems (Sarà et al. 2018). This can be achieved by manipulating biological components of the target organisms, such as through genetic modification or growth hormones in feed, or it can be achieved through the infrastructure of the aquaculture system, such as formulated feeds that cater to specific life histories, physical manipulations, or introducing polyculture. The most direct

approach is to alter the source of feed items, which has been popularized recently in the phrase “Fish In: Fish Out”, which refers to the amount of wild fish required to feed large carnivorous farmed species such as aquaculture (Olsen et al. 2014). The issue of feed inputs ratios is not only applicable to high trophic level carnivores, although that is where the most work must be done but is also to farming invertebrates. In this context, more commonly used phrases include nutrient retention and assimilation, conversion efficiency and biomass conservation.

Nutrient assimilation and conversion efficiency, the processes by which animals turn feed into biomass or other food products, varies by taxa and system of production. Feed Conversion Ratio is a common measure of this efficiency, calculated as the ratio of feed input to biomass increase. FCR values for species of commercial interest reared under intensive conditions (excludes filter-feeding aquatic operations and open-range grazing on land) range from 1.0-2.4 for farmed fish and crustaceans and 1.7-2.0 for chickens (low) to 2.7-5.0 for chickens and 6.0-10.0 for cattle (high) (Table 5). Smaller FCR values represent more efficient systems, and aquaculture typically falls on the lower end of the spectrum. This is partly due to the lower metabolic requirement of cold blooded marine organism who live in a neutrally buoyant environment, necessitating less energy for typical locomotion and feeding than terrestrial organisms (Seibel & Drazen 2007).

Table 5. Summary of current FCR and nutritive content in farmed species adapted from Fry et al. 2018 to include gooseneck barnacles

Species	FCR	Edible portion of animal	Feed content (g or kcal per 100 g feed)		Human nutrition (g or kcal per 100 g serving)	
			Protein	Calories	Protein	Calories
Carps	1.5-2.0	0.36-0.54	17-45	175.8-554.2	18	109-127
Catfish	1.2-2.2	0.35-0.63	28-32	345-390	15-17	117-119
Salmonids	1.2-1.5	0.58-0.88	35.5-44	372-554.5	20	208
Shrimps	--	0.4	25-45	225-433	20	85
Tilapia	1.4-2.4	0.37-0.45	20-32	216-404.4	20	96
Chicken	1.7-2.0	0.7-0.78	18-23	320	18.6	215
Pig	2.7-5.0	0.68-0.76	13.2-20.9	326.5-335.1	15-18.2	211-304
Cattle	6.0-10.0	0.52-0.64	7-15.4	188-339	15-20	214-276
<i>Barnacles</i>	<i>.09 – 1.6</i>	<i>0.21-0.28</i>	<i>49.7-57.3</i>	<i>---</i>	<i>38.1-56.5</i>	<i>---</i>

Table 5. Sources for values presented in this table are as follows: FCR for carps, catfish, shrimp (Tacon & Metian 2008), chicken (Zuidhof et al. 2014), large livestock (Shike 2013); edible portion of animal for fish and shrimp (“Yield and nutritional value of the commercially more important fish species” 1989), livestock (“USDA National Agricultural Library” 2016); protein and calorie content for feed of fish, salmonids and shrimp (“State of World Fisheries and Aquaculture” 2014), terrestrial agricultural species (USDA 2016); protein and calorie content for final commercial product (SR25 2018)(USDA SR25 2018).

Aquaculture with low FCR values has the potential to meet the increasing demand for animal-based protein at the global scale while enacting a smaller toll on the environment (Robinson et al. 2005). The values for gooseneck barnacles presented in Table 5 place this sessile crustacean on the more sustainable end of the spectrum, in both FCR and protein retention. The incredibly low conversion ratio for barnacles fed a rotifer diet (0.008) is especially promising looking into the future. The fish blend yielded a higher FCR but it was well below other types of seafood and similarly sustainable when

compared to other farmed marine invertebrates (Table 5). The proven success of upcycled fish waste in gooseneck barnacle aquaculture is applicable to wider aquaculture. Of all the alternatives to forage fish outline in Table 2, “seafood byproducts” is the cheapest and depending on location, a high volume, high quality source of food. The fish blend diet treatment was originally intended to provide the same nutrition as the natural zooplankton diet available in the intertidal. It was sourced using a variety of tissues from a predatory fish that is at the top of the food chain (tuna). The fish viscera represents marine biomass that is otherwise removed from the marine ecosystem by industry, and is part of a larger narrative around up-cycling industrial waste to minimize larger scale effects on ocean ecosystems from marine industries (Tlusty & Thorsen 2017). If goosenecks can be cultured using this industrial waste not only does the barnacle fishermen benefit from a high quality feed but the fishing industry is able to participate in a sustainable system as well.

This study contributes to the growing body of literature on alternative feed sources in a fairly convincing fashion, however, it is not without confounding aspects. One possible source of error was the variation in particle consistency, buoyancy, and size of the fish blend diet, which resulted in many of the larger lighter particles sticking in the foam at the top of each tank while the larger denser particles sank to the bottom of the tank and thus were removed from circulation and emptied with the waste. The fish viscera were homogenized before being administered to the barnacles in the FCR diet experiment, however, the blend consisted of tissues from multiple organ systems in addition to the more typical “fillet” scraps. These different tissues reacted differently in the turbulent environment of each tank and some were possibly removed with waste each

week without barnacles having consumed them. By the time waste was dried and measured, distinguishing left-over food from biological excrement was uncertain at best. This means that the FCR value for fish blend is potentially compromised by an inexact feed input amount. Coupled with this specific source of error are the broader issues with using FCR as a metric for aquaculture, as it provides limited potential to capture the true efficiencies of a system since it does not account for nutritional quality of the food, edible portion of animal, or nutritional quality of the final product (Torrissen et al. 2011, Fry et al. 2018). These factors typically vary across species, making FCR a potentially flawed measure for inter-taxa comparisons.

FCR is the most widely standardized metric for comparing efficiency across systems and continuing to investigate low-FCR aquaculture is one way to build the body of research that seeks to substantiate sustainable food production practices. Currently the understood paradigm holds that unfed aquaculture, including shellfish and some algae, represents the leading edge of the production of highly nutritious food with low inputs (FAO 2014). The downside of extensive, unfed systems of production is that cultures are dependent on the natural subsidy of food to commercial organisms and are susceptible to fluctuations in oceanographic conditions that could be potentially detrimental. The rotifer-fed gooseneck barnacles displayed a range of FCRs that were so low as to approach the zero of unfed systems, with the additional benefit of being contained in a system where every variable is accounted for and the environment can be controlled directly to accommodate other biological needs. These factors alone qualify *Pollicipes polymerus* as an ideal candidate for scaled up commercial aquaculture. In addition, the peduncle of the gooseneck barnacle has a higher protein by weight than other sources of

nutrition from marine or terrestrial environments (Table 5). One avenue of research that was not explored in this study but that would provide complimentary data for commercial fisheries interested in gooseneck barnacles is to investigate the fatty acid profiles and potential bioconversion abilities of this species. Fatty acids are a topic of emerging interest in human nutrition and as such there is incentive to study fatty acids in aquaculture, particularly sustainable aquaculture such as low-trophic level benthic invertebrates.

Unfortunately, Gooseneck barnacles are more difficult than other sessile invertebrates to culture and grow from spat, and do not thrive in natural environments except where they are exposed to heavy wave turbulence, which limits the areas available for grow out. These biological constraints limit the potential for scaling up, if gooseneck barnacles are the only species in a culture system. However, polyculture, the simultaneous co-cultured of two complimentary species, of gooseneck barnacles would likely be easier to scale up and more economically feasible. There is increasing interest in polyculture, particularly if the organisms provide an ecosystem service in addition to a commercially valuable product. Polyculture operations that incorporate components interacting at the ecosystem level, rather than population or individual level, have become more popular in recent years with the first multi-species offshore aquaculture facility in federal waters off the coast of southern California. Moving forward with gooseneck barnacle aquaculture, future research might investigate the polyculture potential of growing barnacles in a well-aerated system alongside fellow sessile invertebrates with similar commercial value such as abalone and mussels.

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