Final Report

Beneficial Environmental Effects of Marine Finfish Mariculture

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Acknowledgments

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Executive summary

A study to quantify the types and volumes of biocolonization at a commercial net-pen fish farm site in North Puget Sound Washington was conducted in 2004-2006. The algae and invertebrates that colonize nets, walkway floats and anchor lines have rarely been studied in detail, yet it is an economically-important problem to fish growers as they can foul the nets, reduce water flow through the pens and necessitate frequent cleaning and maintenance.

This study shows that a typical floating fish pen system in Puget Sound is populated by a diverse group of over 100 species of seaweeds or invertebrates. These species provide a locally important component of the food web, providing enrichment for a variety of marine food web life including marine bird species. In this regard, the biofouling can be considered a “beneficial” effect of fish farming if we value diverse and richly-populated marine food webs. The popular media-distributed notion of fish farming habitats often suggests a biological wasteland, heavily impacted by fish feces, waste feed, antibiotics and chemicals. Nothing could be further from the truth for Washington State fish farms (and those in the State of Maine). Antibiotics are rarely used (vaccines are used instead), no sea lice problems exist due to naturally reduced salinity levels, and farm siting involves locations with fast currents or relatively great depth that distribute wastes over large areas where they may be incorporated into the food web while maintaining aerobic surficial sea bottom sediments.

The flora and fauna of the subject net pens did not include any harmful, invasive exotic species (e.g., exotic tunicates) and was not similar to that seen on floats and pilings in degraded, marina environments in urbanized bays and marinas. Rather they included a diverse assemblage of species, many of which could be considered important prey items in the food web. This result should not be surprising, as net-pen siting and operational practices in the Pacific Northwest have evolved greatly from their beginnings over 30 years ago from backwater locations to fast flushing, nutrient replete channels with good water quality.

Fish containment nets provided over 18,000 m^2 of submersed surface area (one side only), far exceeding the submerged area of anchor lines and walkway floats. Approximately 360,000 individual invertebrates were collected, identified, enumerated, weighed and recorded on these surface areas. Thousands of seaweed samples were collected too. At least 100 species were identified and the total number of species probably far exceeds that number as some major groups like polychaete worms were lumped into major groups only due to the sheer volume of samples. About 1/3 of the species diversity was represented by seaweed or algae, the other 2/3 were invertebrates and three species of fish.

The quantity of biofouling existing on the submerged surfaces during summer was measured to be approximately 55 metric tons (wet weight, 95% confidence interval of 45.6 to 65.5 MT). This is a large amount of biological material in a small area, but represents only about 5% of the peak farmed fish biomass held at one time during the study period.

The single most important substrate was anchor lines (23.8 MT) due principally to the presence of an ecologically desirable species of seaweed known as bull kelp (*Nereocystis luetkeana* about 2/3 of the wet weight of all colonizing species) but also to the fact that anchor lines are a stable habitat for many years before they are routinely replaced, allowing a climax community of kelp, tube worms and other species to become well established. This was a surprising result because the submerged surface area of anchor lines was only 0.6% of the total, the bulk of it was nets (90.5%) and walkway floats (8.9%). Walkway floats and fish containment nets had equal biovolume of invertebrates and algae (15.9 MT each) during summer but very different species compositions. Barnacles were not numerically dominant but because of their heavy shells were important by volume. Mussels (*Mytilus*), the seaweed *Costaria costata* (five rib kelp) and sea anemones (*Metridium senile*) and ribbon kelp (*Alaria marginata*) were 2nd through 5th important for wet weight biomass, respectively on walkway floats. Biocolonization on nets was dominated in spring by massive abundance of pink-top hydroids (*Ectopleura marina*), mussels and amphipods but shifted to caprellid shrimp, filamentous diatoms/algae and amphipods in both summer and winter. The biofouling was on nets that were periodically cleaned, sampling sites were selected randomly. The quantities of caprellid shrimp and amphipods on the netting was incredibly large (several hundred thousand per m^2) which made sample sorting and enumeration very difficult. Special techniques were developed to deal with these challenges.

In addition to a diverse assemblage of organisms, some of the most abundant were also commercially important species or potentially important. Mussels (*Mytilus edulis*) were common on floats, nets and lines despite intense predation by sea ducks (surf scooters) during the fall through spring period. A seaweed of potential commercial value, *Alaria marginata* (ribbon kelp), was the second most dominant contributor to biomass on anchor lines during summer, after bull kelp. Seaweed and mussels are being grown in other regions to sequester wastes from net pens as a means of mitigation known as Integrated Multitrophic Aquaculture (IMTA) or simply Integrated Aquaculture. Such a technique may be of benefit for some sites in the Pacific Northwest too, although most sites in Puget Sound have strong currents and all sites are in non-nutrient sensitive areas so there is no existing problem with nutrients or solids assimilation into the food web. It is possible to position mussel rafts or kelp-growing lines downstream of some net pens without compromising water flow rates that maintain oxygen concentrations or strong subsurface waste resuspension rates.

Naturally occurring stable isotopes of nitrogen and carbon were measured in several key biocolonization species at the farm site and at a reference area. Enrichment of the ratio of ^{15}N to ^{14}N isotope form of the nitrogen at the net pen site is the basis for the measurements. These data indicate a direct positive transfer of fish farm nitrogen to several key organism including amphipods, caprellid shrimp and mussels on nets and floats. A positive effect was also measured for carbon isotope transfer to mussels. *Metridium senile* (common plumose anemone) results did not indicate a stable isotope signal; they may not benefit as local research has shown they feed exclusively on pelagic zooplankton. The farm walkway floats provide habitat for them, but apparently not feed. Other species remain to be investigated regarding their stable isotope signature including bull kelp.

By further sampling of feed and fish feces it may be possible to quantify the degree to which each key biocolonization species is benefiting from farm wastes through application of a standard mixing model. Other studies could include assaying the effects on small fishes that sometimes reside downstream of the farms or on sea ducks who obviously are attracted to the farms and nearby enriched habitat. This study is only one contribution to the beginning of our understanding of the extent of food web enhancement that likely exist at most properly sited and operated fish farms.

1. Introduction

Marine fish farms are subject to colonization by a number of species of marine invertebrate, seaweeds and other organisms. A fish farm in North Puget Sound in Washington State is the focus of this report, where we characterized the species composition and biomass of biocolonization on nets, floats and anchor lines. Most of the effort was directed at determining "standing stock" conditions (i.e., volume of colonizing organisms in different time periods), although some effort was directed at determining rates of colonization. Stable isotopes of carbon and nitrogen were assessed in biota too, as the ratio of these isotopes to normal isotopes can be used in some circumstances as a tracer of fish farm waste use by nearby animals and plants.

Thousands of studies have focused on the benthic and water column effects of such fish farms, but very few have involved the colonizing invertebrates and plants associated with such farms. The studies demonstrate that proper siting is a key factor to both success of the farms and maintenance of a diverse and productive surrounding environment. The colonizing biota on fish farms is certainly of economic interest, as cleaning of nets is in some cases a significant cost and use of antifoulant on netting has raised concerns. From other perspectives, however, biocolonization is of ecological interest as floating fish farms potentially represent significant habitat for species that could be beneficial or harmful to the surrounding regions' biota and food web.

Floating net pens regulation

The first sea cages used in coastal waters were installed in the late 1960s or early 1970s in several locations worldwide, and since then commercial-scale fish farming has expanded in many countries and advanced rapidly in terms of efficiency and reduction of environmental effect. Growth of U.S.-based net pen farms has been minimal since the 1980s in part due to opposition from shoreline owners, nongovernmental organizations, and traditional fishers and their organizations. The reasons for opposition are varied, ranging from perceived environmental risks such as water or sediment quality effects, escapes, disease and aesthetics but also include fear of economic competition. This section briefly summarizes the scales of effects of salmon net pens in Washington State, their regulations, performance standards, and recent advances in environmental performance.

Fish farms in Washington State are allowed a maximum sediment impact zone of 30 m distance from the cage perimeter. At the edge of this zone, background conditions must match remote reference or background conditions. This is a remarkable decrease in extent of effect, as at least one atypically large fish farm (the largest ever assembled) studied by Weston (1990) had adverse benthic effects to ~5 times past the present spatial limit in the prevalent downstream direction. Present day facilities are sited in areas that have no resemblance to the poorly flushed, nutrient sensitive bays where net pen mariculture began over 35 years ago.

The Washington State NPDES net pen regulatory program is administered by the Washington Department of Ecology. The program draws on two decades experience of monitoring, analyses and fine tuning. Net pens are required to meet screening-level physicochemical standards including surficial sediment total organic carbon (TOC) that is compared to background conditions. TOC is important because fish feces and wasted fish feed contain carbon that demand oxygen during bacterial and food web respiration and assimilation. Strong currents allow dispersal of the wastes and aerobic assimilation but weaker currents may result in anaerobic surficial conditions that can reduce benthic invertebrate diversity. Farms must meet background levels of TOC at the SIZ perimeter or the sampling must be broadened to include detailed measurement of benthic invertebrates (infauna) and performance standards that apply to all industries and municipalities discharging into marine waters of the state. This is an over-simplification of the entire performance-regulatory process for Washington State net pens; see other references cited below or contact the Department of Ecology for more details.

Presently all eight commercial net pen sites meet performance standards discussed above. A few of the sites had borderline results several years ago but the farmers responded quickly by modifying the pen configuration or operations to reduce the effects. Subsequent sampling showed a reduction of effect. The net pen rules and performance standards are adjusted every five years when the NPDES permits are re-issued, but for three permit cycles the above TOC core sampling has remained. Sampling of sediment copper and zinc has been added to the monitoring protocols (due to copper antifouling use and zinc for fish dietary supplementation) but sampling has shown essentially no measurable effects of either. Nets are received with copper treatment when new, but are not retreated subsequently. Dissolved oxygen is routinely monitored and is reported to the Department of Ecology from near the cages and a reference area. Measurable effects are typically found only a few meters downstream and never 30 m or beyond.

Finfish aquaculture

Each set of commercial salmon pens in Washington State produces several million pounds of salmon for U.S. seafood market while paying significant lease rates to the State Department of Natural Resources. Yet as mentioned above, measurable "adverse effects"[1] on the sea floor benthos are limited to beneath the one to two acre dimensions of each farm and immediately adjacent seafloor. To meet the strict performance standards and retain all effects within a 30 m perimeter (the impact zone limit both in Washington State and Maine) the degree of effect immediately under the cages must be minimal. Some species of invertebrates may be extirpated, others are enhanced and no pens in the U.S. cause "azoic" conditions, or loss of all sediment-dwelling invertebrates beneath them.

Limitation of the extent of effect has been achieved by an evolution in siting and operation of the farms over the past 35+ years. This involves a shift to sites with greater current velocity, to disperse the organic wastes over larger areas while improving fish retention of feed. Some would argue that no effects of the fish farms should be allowed, but the temporal and spatial impacts of floating fish farms are short term (complete recovery of the sea floor biota in relatively short periods) and limited in magnitude.

Worldwide seafood demand is fast outstripping the ability of wild fisheries to satisfy the need and aquaculture production is increasing rapidly as a result. The U.S. imports a huge amount of seafood annually, in excess of US$ 10 billion annually with a value second only to petroleum products. Concurrently, there are serious concerns about food safety from some exporting economies. The solution to this situation is for the U.S. to produce more seafood for its own use, but wild fisheries

[1] Adverse effects include reduction in the numbers or diversity of benthic infauna (e.g., worms, molluscs or crustaceans) although total biomass often increases and in some cases adverse effects are not measurable due to strong currents. Often epifauna and demersal (on the bottom or immediately above the bottom) animals such as shrimp or crabs increase significantly as a result of increased food supply and shading of the bottom.

are fully or indeed in some cases, overexploited. Marine fish farms in North America and elsewhere are often accused of being ecological problems, creating dead zones beneath them, introducing antibiotics and chemicals into the sea, causing losses or contamination of wildlife, etc. There were some significant issues with the first salmon farms in Puget Sound and elsewhere, but the earlier criticisms have, with few exceptions, been addressed or mitigated through improved siting, operation, monitoring and regulatory practices. See WDF (1991) for more on this topic with more recent updates by Nash (2001) Rensel (2001), Rensel and Whyte (2003), Brooks and Mahnken (2003) and references within these documents for additional details.

The flux of waste products from salmon net pens and its nearfield fate is fairly well understood at this point. Much of the waste material from modern salmon farms is associated with the fish feces, which includes biologically labile carbon and phosphorus components. Nitrogen wastes from salmon farms are much less than carbon and associated with the dissolved urine wastes, ammonia and much smaller amounts of urea that both are typically oxidized rapidly to nitrate nitrogen. In the Pacific Northwest and in all cases in Washington State, marine fish farms are located in areas where nitrogen is naturally abundant due to oceanic input. These areas, termed as “nutrient insensitive” are where sunlight, not nutrients, control the amount and growth rate of microalgae (phytoplankton) and macroalgae (seaweeds).

Figure 1. Collection of diverse invertebrates populating the undersides of a walkway float at the Deepwater Bay net pens.

While we believe that the detrimental impacts of existing, properly located commercial net pens in the U.S. are far overstated by some interest groups, it is not surprising that this has occurred. Previous studies have often focused on exceedingly large (e.g., Weston 1991) or exceedingly poorly flushed sites (e.g., Weston et al. 1994) that no longer exist in the latter case or have been significantly reconfigured in the former. There is also an economic interest in preventing net pen expansion in the U.S. Wild fish capture advocates fear the economic competition and NGO groups find funds from foundations and contributors to oppose expansion. As noted above, most of the problems associated with net pen aquaculture in the U.S. are manageable or have been dealt with in great detail by responsible government authorities and progress by industry, but the popular media is not discriminating in its use of source materials and keeps the spotlight on the supposed adverse effects.

There is a need to move beyond this obsession with potential adverse effects and turn our attention to positive ecological effects of fish farming. As discussed below, these effects are real but more difficult to document than near field benthic effects as they can involve movement of nutrients through several trophic levels of the marine food to higher and more mobile organisms such as seabirds or fishes. Unlike sedentary benthic invertebrates, these mobile organisms are by nature more difficult to study using traditional marine biological techniques and metrics.

If there is ever to be a significant increase in U.S. mariculture production, either nearshore or offshore, U.S. scientists, regulators and consumers must be made aware of the true nature of the industry as it presently exists, including the potentially positive food web and biodiversity aspects of net pen farm structures. We make no other comment on the supposed differences between inshore or offshore net pens except to note that the rules of physics and biology apply in both locations. Weak currents in either case can limit fish production in the cages (due to limited oxygen supply) or have adverse effects on the sea bottom. It is our opinion that there are suitable and unsuitable locations for both and the rules and experience of inshore aquaculture must not be ignored if offshore aquaculture is to be successful and ecologically sound.

NOAA has proposed a two million ton increase in annual production of U.S. seafood from mariculture by the year 2025. This goal will not be met without political support and understanding and, just as importantly, the publication and dissemination of results that can be used to refute some of the current misconceptions and misinformation of net pens as highly-polluted, non-sustainable food production systems.

The positive aspects of marine fish pen operation on the benthos have been documented in a few cases, i.e., the bio-stimulation of invertebrates in the sea bottom. This is the “halo effect” of carbon enrichment surrounding fish farms that can lead to increased diversity of species and biomass abundance when the waste load is balanced with the assimilative capacity of the food web (Pearson and Rosenberg 1978). This halo effect does not always seem to be present, but perhaps this is due to the type of sampling that is often conducted which focuses on adverse effects nearest to the pens, when it occurs. It may also be an artifact of the usual focus on benthic infauna sampling that is relatively easy for soft substrates but coarse substrates with resuspensional sea bottoms are often only documented by photographs. Also it is rare to examine other components of the food web. We believe that the organic enrichment that properly sited fish farms provide flows throughout the food web in the vicinity and in some cases beyond. As techniques such as stable isotope tracing advance, the nature of these flows will be documented.

Fish farm habitat and biocolonization

The habitat-creation effect of the floats, nets and lines associated with net pens is sometimes cited (e.g., Nash 2001) but to the best of our knowledge, never quantified in detail. Most prior environmental work regarding net pen effects in the U.S. has concentrated on adverse benthic effects (see reviews by Weston 1986, WDF 1991, EAO 1997, Nash 2001) and with few exceptions, facilities selected for peer-reviewed publication study were atypically large (e.g., Clam Bay net pens of early 1980’s, see Weston 1990) or atypically very poorly flushed (antibiotic residue studies in Port Townsend Harbor, Weston et al. 1994). Nutrient effects from salmon net pens are less important presently in the U.S. as most commercial salmon net pens on both coasts are located in non-nutrient sensitive areas (Rensel Associates and PTI 1991, Rensel 2001, Rensel and Whyte 2003, Normandeau Associates and Battelle 2003).

Fish growers typically consider the invertebrates, algae and seaweed that grow on their facilities a potentially dangerous nuisance, as it can restrict inflowing oxygen and waste distribution out of the pens. Extensive costs are associated with removing and cleaning the netting of the cages. Automated cleaning of nets has been proposed and some initial designs developed, but farms rely on manual cleaning methods. Modeling of the effects of fish farms by necessity has ignored the effects of the colonizing organisms, simply because the extent and function of these species has not been measured.

Study Design Overview

This study was undertaken at a relatively large Atlantic salmon (*Salmo salar*) farm that had been in continuous production since 1991.

This study had five major components and goals included quantification of:

1. **Standing stock** (wet and dry biomass) and species diversity of invertebrates and plants on nets, floats and anchor lines of a commercial fish farm in Puget Sound or approaches.
2. **Colonization rates** on cleaned nets and floats over a two year period both at the farm site and at a reference site nearby, but outside the direct effects of the waste plume of the farm.
3. **Stable isotope content** of dominant invertebrate and plants to indicate the probable enhancement effect of wastes entering the food web.
4. **Seabird use** of the surrounding area and why the fish farm studied is likely helping support these birds through enhancement of the food web.
5. **Photograph the biofouling succession** that occurs on nets and floats at the same treatment and reference sites noted above.

Goals 1 through 4 were achieved to varying extents as reported here, but due to budget cuts of about 50% at the initiation of the project goal 5 was completed to the extent of measuring the rate of colonization on floats and taking periodic photos designed for area assessment. Goal 3, the stable isotope assessment was partially completed and requires relatively limited studies for conclusion. An additional goal to document the wild fish distribution near the farm was not attempted due to the budget cuts, although it could be done in subsequent studies.

2 Biocolonization at Net-pen Farms: Literature Review

The scant literature involving invertebrate and algal growth on temperate water fish farms is briefly reviewed in this section. Some initial work has been done on this topic for tropical fish farms, but that is not reviewed here.

Marine fouling organisms were studied extensively in the past century, but usually in relationship to specific situations such as boat hulls, pilings, drilling platforms and water intake structures (Railkin 2004). Most of the research on these communities has been directed towards preventing recruitment or eradicating established communities, as they can cause damage to structures, in the case of boring organism or impede vessel progress and increase energy costs.

The few estimates of total net pen fouling biomass weight were unsubstantiated, and in one case suggested a doubling of total weight of the net pen itself (Beveridge 1987). Moring and Moring (1975) conducted the first assessment of net-pen biofouling in Puget Sound, but it was restricted to netting only, was only conducted for part of one year and focused on very small non-commercial pens of much smaller mesh size texture than those used presently for commercial culture. Taxonomy was limited to higher levels only but nevertheless, this study provided a valuable benchmark for comparison for the present study.

In British waters, Milne (1970) provided some information on this topic, but mainly related to fouling resistance and drag force on nets. Methods, intermediate calculations and assumptions were not clearly stated in the latter study and included no species information. Inoue (1972) measured flow rates of flow through and around nets that also relate to the degree of invertebrate and algal colonization but this was not measured or discussed.

Cultured spot prawns (*Pandalus platyceros*) were effective in removing many types of marine fouling organisms from net pens that concurrently contained grower-sized salmon (Rensel and Prentice 1978, 1979) at the same location studied by Moring and Moring (1975). At that time spot prawn culture was being considered as a companion crop to Pacific salmon or as a means to enhance wild populations. Photographs of treatment versus reference panels of netting clearly show the effect of the prawns' foraging, but no quantitative estimates of biomass were conducted.

Sayer et al. (2002) found approximately 40 different taxa in a study of biofiltration near salmon farms in the lochs of Scotland with hydroids, tunicates and bivalves included among the dominant species. The potential for biofiltration as mitigation for removal of waste products of aquaculture has been recognized by some (e.g., Hughes et al. 2005). These authors note there are few quantitative estimates of the effectiveness of the biofiltration but they provide some probable scale of effect. They conclude that the costs of actively providing biofiltration substrate would exceed the benefits while noting that siting has a huge effect on the calculations (Cook et al. 2002).

The same group (Cook et al. 2006) conducted extensive measurements on artificial structures placed near salmon farms in Scotland (N = 2) and marine fish farms in the Mediterranean Sea (N=4). They also concluded that introducing artificial substrate in these areas to remove significant amounts of fish farm effluent would not be cost effective, but still might be considered where removal of a small percentage of wastes would be important. This important article is discussed in more detail later. For now it is sufficient to offer that the areas studied by these authors were in all four cases likely to be nutrient sensitive, i.e., additions of dissolved inorganic macronutrients would result in uptake by algae in relatively rapid fashion. This situation is different than commercial fish farm sites in Puget Sound and the Strait of Juan de Fuca region where light, not nutrients, is the controlling factor. This distinction is made to underscore the point that eutrophication of local waters in Puget Sound is highly unlikely from well-placed net pen aquaculture, as discussed above.

Biofouling at a submerged net pen site in the tropical waters offshore of Puerto Rico was studied as part of a larger study by Alston et al. (2005). The authors examined biofouling on small sections of netting that were attached over the net pen netting both above and below the perimeter ring of these SeaStation systems, which allowed recovery without damaging the nets. They then photographed the net samples and used software to determine "percent coverage" of the biota that covered the netting. The experimental design was for the nets to remain uncleaned, but biweekly to monthly cleaning of the pens may have affected the test sections. Coverage of biofouling on the nets was relatively rapid with up to 50% coverage in two months at both of two sampled cages. There were seasonal changes in major taxa composition, with seaweeds ranging from near 20% to more than 60% of the coverage during the 5 sampling periods. Upward facing surfaces had a higher percentage algal covering than those shaded and below. Other dominant taxa groups included hydroids, small mollusks and ascidians (tunicates) as well as fewer bryozoans and sponges. The authors noted that biofouling could be a serious limiting factor to fish production if cleaning was not routine.

Many prior marine fouling studies of the surfaces of pilings or docks in ports and marinas are also not applicable to commercial fish farms in North America and particularly in Puget Sound. These farms presently occupy locations with excellent water quality, compared to the bays and inlets where marinas and ports are presently located. This was not always the case, Washington State fish farms have evolved from back water bays and inlets to well flushed channels and bights (Rensel 2001). Fish farms generally do not remove fouling organisms from floats and rarely do so for anchor lines, except during routine maintenance, allowing for accumulation of large biomass and possible succession of species assemblages. Pacific salmon reared in net pens generally do not consume biofouling organisms associated with pens, as first studied by Moring and Moring (1975). The same is likely true for Atlantic salmon.

Small and large wild fish are often associated with any floating structure in the ocean, and net pens are no exception. In Puget Sound these fishes may include sand lance, herring, perch, and smelt, but no inventories of any kind have ever been performed for these structures. In the past there was concern that antibiotics used by fish growers could be passed on to these fishes, but this is less of a concern presently, as salmon are immunized for disease prevention. Antibiotic use has declined exponentially over the past decade or more, and is currently very low compared to other agricultural sectors (MacMillian et al. 2003). It is not uncommon for Puget Sound fish farms to operate for years without the use of antibiotics, and all therapeutant use is reported as required by NPDES permits.

Most prior environmental work regarding net pen effects in the U.S. and abroad has concentrated on adverse benthic effects (see reviews by Weston 1986, WDF 1991, EAO 1997, Nash 2001) and with few exceptions, facilities selected for peer-reviewed publication study were atypically large (e.g., Clam Bay net pens of early 1980's, see Weston 1990) or atypically very poorly flushed (antibiotic residue studies in Port Townsend Harbor by same author). Nutrient effects of salmonid aquaculture in the U.S. are less important or not issues at all as most commercial salmon net pens on both coasts are located in non-nutrient sensitive areas (Rensel Associates and PTI 1991, Rensel 2001, Rensel and Whyte 2003, Normandeau Associates and Battelle 2003).

Recently some critics of aquaculture have interpreted experimental work by two workers at the Washington State Department of Ecology (Newton and Van Voorhis 2003) as indicating that Puget Sound is nutrient sensitive and that limits to nutrients should be established. The interpretation was from a bottle experiment where 30 uM ammonia was added to seawater from a number of locations in or around the fringes of the main basin of Puget Sound. The critics interpretation is ill advised and incorrect. The authors of the study repeatedly state that the bottle experiments did not allow for the process of zooplankton grazing which is a major control to phytoplankton production. Nor was it noted that managers have long known that there is considerable temporal and spatial variation in nutrient sensitivity and that some subareas have to be protected from all anthropogenic discharges. I would rapidly add that a decade of repeated up and downstream monitoring of nutrients around fish farms in Puget Sound shows that very little ammonia is observed downstream (having been oxidized to nitrate) and that by 30 m downstream the level of nitrate, nitrite and total ammonia (DIN) is highly diluted so as to be only a few tenths of a micromole above ambient. These are not conditions likely to stimulate algal blooms of any kind given the cell reproduction times of microalgae (a day or more) even if ambient nitrogen concentrations were low. Rensel (2007) addresses these points in more detail with regard to harmful algae studies in Puget Sound.

It should be noted that there are no salmon farms near the study locations and that all of the locations were remote from the typical channel environment where fish farms are located that have relatively strong vertical mixing all year and thus high natural DIN levels. Add to that tremendous within and among year variation as well as spatial variation in the results and the fact that bottle experiments are notorious for producing potentially unreliable results, and the conclusion that all of Puget Sound is nutrient sensitive is hardly warranted. This study is not a repudiation of the long held concept that phytoplankton production in Puget Sound is mostly limited by light during the entire year. The one station that exhibited the most sensitivity was near a river mouth where stratification is induced to allow spring blooms and short term nutrient depletion. None of the findings repudiate the basic concept that if naturally occurring levels of DIN are about some moderate threshold (about 2 to 6 uM) then no amount of additional nutrient will lead to additional productivity by the phytoplankton in that area. Degree of nutrient depletion of surface waters in combination with dissolved oxygen depletion have been the basis of salmon net pen “zoning” for over 20 years (SAIC 1986) and subsequent evaluations of Puget Sound nutrient sensitivity (Rensel Associates and PTI 1991 and subsequent Dept. of Ecology publications).

Positive food web aspects of marine fish pen operation on the benthos has been know of for many years, but not well documented. Bio-stimulation of invertebrates in the sea bottom or a “halo effect” of carbon enrichment surrounding many fish farms that can lead to increased diversity of species and biomass abundance has been documented (Pearson and Rosenberg 1978, Barnett 1991, other fish farm effect references cited herein), but the effect is not always demonstrable or sampling is purposely biased to the area immediately beneath and adjacent to the farms where adverse effects are more likely. The floating-reef, habitat-creation effect of the floats, nets and lines associated with net pens is sometimes cited (e.g., Nash 2001) and to the best of our knowledge, never well investigated or quantified in temperate waters and nowhere quantified in terms of biovolume.

3. Site Description

The main study site was in Deepwater Bay, North Puget Sound, adjacent to Cypress Island in an area with three sets of pens in one subarea (Figure 2). This area is typical of Puget Sound net-pen sites with salinity of ~26 to 30 psu, water temperatures ranging from 8.0 to 14.5° C, high natural levels of dissolved inorganic nitrogen normally much greater than about 10 uM, and a relatively coarse bottom associated with fast currents (Rensel and Forster 2003, Rensel et al. 2006). The site is operated by American Gold Seafoods under an aquatic lands lease with the Washington Department of Natural Resources and has been in continuous production for over 20 years.

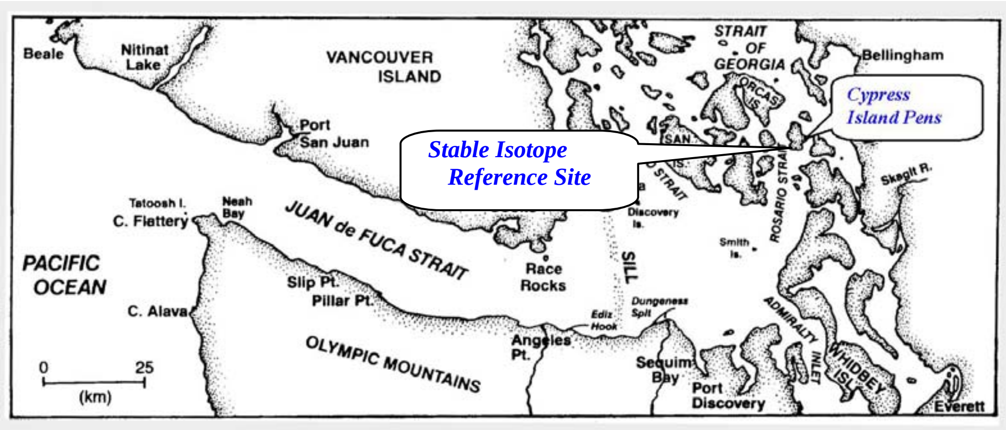


Figure 2. Project vicinity map (above) and aerial photograph of all three AGS sites at Deepwater Bay, Cypress Island with arrow pointing toward Site 3 (right, photo by K. Bright).

The study site (known as "Site 3" by the fish farm company) is in an area of moderately strong currents that was previously documented by Rensel (1995) on a complete ebb and flood tide during near-average tidal-amplitude exchange. Mean velocity was 22 cm sec^{-1} at 2 m depth and peak velocities exceeding 75 cm sec^{-1} during spring tides. This site was selected because other sites in the area were scheduled to be harvested and left fallow during a change in company ownership.

Site 3 was monitored annually for benthic effects of the pens from 1986 to 1996 as reported to state agencies including WDNR. After 1986 the site was monitored under NPDES permit regulations as reported to the Washington Dept. of Ecology. Bottom sampling has been limited to video recordings as the currents are so strong that grab sampling of the coarse sand, rock and shell bottom is not feasible. Research studies have occurred here too, including various studies of the occurrence and mitigation of harmful algal blooms that are advected into the area from remote locations (Rensel et al. 2003).

Biomass of farmed salmon averaged 593 MT and peaked at 1080 MT during the duration of the study (Figure 3), dropping to zero for a short period in spring of 2005 due to harvest and a change in company ownership. The site has operated since 1986 with biomass usually exceeding the values seen during this study. Much of this portion of the study was completed in summer 2004 and winter of 2004-05 prior to the decline in fish stock.

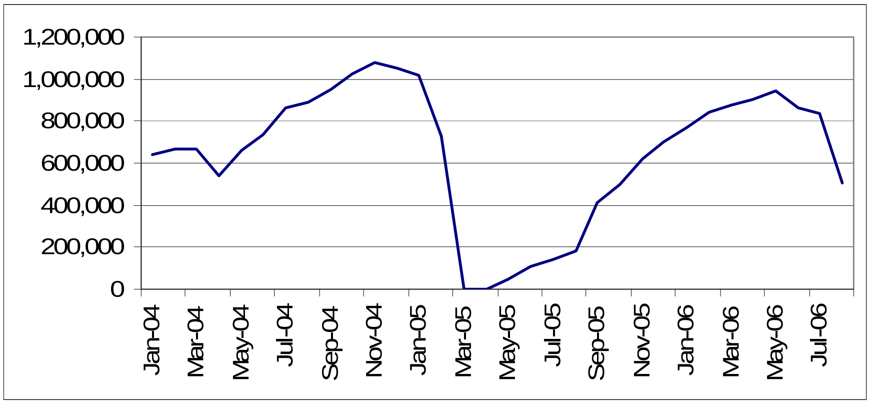


Figure 3. Fish stock biomass in kg during the study.

Two reference areas were used for obtaining specimens. For the stable isotope study, very small quantities of invertebrates or algae were collected from anchor buoys near the north end of Cypress island were used. The reference area shares a similar water source and flow conditions as Deepwater Bay but is hydrodynamically "upstream" of the pens in terms of the estuarine outflow in the area caused by major rivers to the north. For the standing stock and colonization study we elected to use an area much closer to the pens to avoid any difference in biogeography such as larval recruitment. This area was 50 meters SE of the pens in a direction that was always upstream from the cages based on current meter results (Figure 4, Rensel 1995) and observations of long-time farm staff.

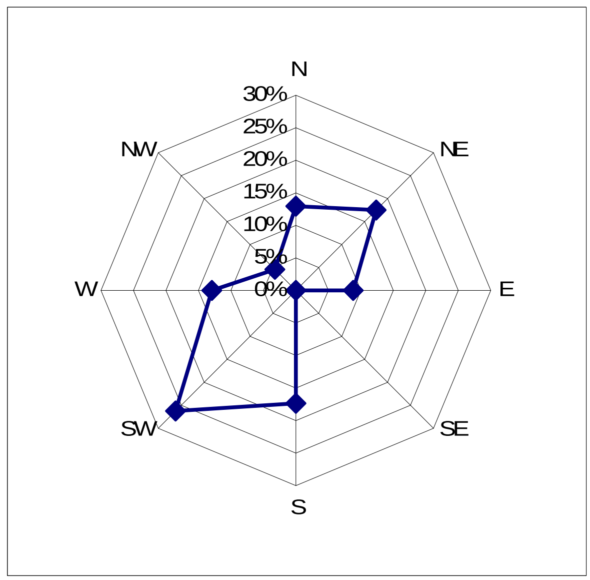


Figure 4. Water current direction rose indicating percentage of time current flows in any one compass direction for subject site (Rensel 1995).

4. Methods

Collection and transport methods

This section includes an overview of the standard methods used to conduct this study. Special attention was given to watching for unusual or rare species as well as exotic species. Several recent studies in Puget Sound were consulted in this regard (e.g., Cohen et al. 1998). The primary author has extensive prior experience with marine invertebrate identification, but an effort was made to obtain and use all the latest guides, keys and references as taxonomy is a moving target.

Floats

For the standing stock assessment, floats were selected from either end of the up and down current, longitudinal ends of the fish farm. Other floats were inaccessible, a factor that is discussed later in the results and discussion. Typical net-pen floats were disconnected by removing mounting bolts, then the net-pen walkway lifted with a ship's crane, and the float was allowed to slip out sideways. It was lifted carefully with a pallet fork (Fig. 5) and placing the float upon a plastic tarp for photography and sample removal. Each side and the bottom were photographed then a 0.25 m^2 pipe and string frame was placed randomly on or alongside the surface to sample. A single sample was removed from each of the four sides and one or two samples from the bottom. A clean paint removal scraper was used to remove all organisms or debris in the subsample area that was then placed in labeled zip lock bags and placed on ice immediately.

Figure 5. Removal of cage float for sampling by Bill Clark, AGS Inc. Deepwater Bay site manager.

For the colonization assessment, we completely removed all organisms and debris from both reference and treatment floats in May 2004. Treatment floats were in the same general area of the standing stock assessment floats discussed above and were cleaned *in situ* by using paint scraper and a commercial power washer operated by a diver.

Figure 6. Sampling by scraping biocolonization from different surfaces of a net pen float.

For reference floats, we removed an entire 12m long net pen walkway from the water, inverted it to face bottom up, scraped and power washed all six plastic floats of biofouling materials and allowed the assembly to completely dry for 1 week. The walkway was then re-floated and installed 20 m southeast of Site 3, in an area which is always upstream of the cages (based on current meter data and decades of farm-staff observations). Monthly photographs were taken of all colonization substrate in the summer and fall and semimonthly in the winter, from all sides, immediately upon removal and before random selection of subareas to be sampled. Distance to the substrate was controlled by use of a stainless steel rod attached to the camera, but strong currents at times made photography difficult.

Nets

Nets were sampled by pulling up a portion of the web onto the pen inner rails, arranging the netting so the bars were evenly arrayed and using a razor blade knife to cut out a square section of netting after counting the number of mesh bars. Typically we sampled at 1.0 m depth, although two sets of samples were taken from 1, 5, and 10 m depth concurrently to determine depth differences if any.

Figure 7. The primary author removing a panel of netting from a heavily fouled net.

Figure 8. Henry Valz sewing in a replacement patch of netting from a one meter deep net panel.

Lines

Sampling of lines was limited to existing lines that had been in place for differing numbers of years. Initially we placed new anchor lines near the ends of the fish farm to provide colonization substrate and we began following the biocolonization process through periodic underwater photography at specific depths. Subsequently these lines were accidentally damaged during fish farm operations so we only assessed standing stock of organisms on lines that were removed in summer 2004 during anchor line removal for maintenance. We subsampled several sections of the lines by cutting out 20 cm sections of the 4 cm diameter line. The line was placed in plastic bags and iced for analysis within two days, as per the other types of samples. No data were collected for line colonization, but a year's worth of monthly photographs were taken before the lines were damaged.

Summer sampling of bull kelp (*Nereocystis luetkeana*) was conducted by subsampling lines on all sides of net pen. All living plants were counted on August 7th 2004 from a small boat on all 29 principal anchor lines. A total of 76 of the counted 3,064 plants were removed and weighed wet in total including the holdfast as well as after drying in a laboratory oven. As the sampled plants were sampled in a stratified (by side of the pen system) random fashion the results were applied to the total count to estimate the total wet and dry biomass.

Sample Transport and Processing

All samples for biocolonization assessment were collected live, placed in large ziplock bags with seawater and transferred in iced coolers to a refrigerator for storage at 5°C. Samples were sorted within 3 days (usually 1 to 2 days) and identified using several guides and keys including Kozloff (1999), Kozloff (1983), Druehl (2000), Mondragon and Mondragon (2003), and Waaland (1977). Sorting, identification, enumeration and weighing were conducted within 1 to 3 days in all cases, usually within 2 days on average. Individual taxa or species were sorted into separate containers, blotted dry with paper tissue, placed in aluminum weighing boats, weighed to within 1 mg and dried in a laboratory oven at 103°C for 24 hours. Some very numerous taxa (e.g., amphipods or caprellids on nets) were often subsampled by separating all of them by forceps or sieves, removing 100 or more for a subsample to be counted and dried and using the ratio of dry weight of the subsampled portion to the dry weight of the remaining portion to estimate total number. Dried specimens were removed and immediately weighed again for dry weight. Calcareous species were weighed with their shells or shell fragments. Some of the dried organisms were placed in whirl-pac bag and frozen for stable isotope analysis described below.

Colonization Assessment

An attempt was made to quantitatively measure the rates of colonization on treatment and reference nets, floats and lines.

In May 2004 we carefully cleaned large sections of regular net pen containment netting using power washers while diving. The sections were from top to bottom of the netting, with a width of two meters. At the same time, six large walkway floats were removed from the pens, scraped clean and dried and reinstalled. On the day of reinstallation, a separate reference 10 m long float walkway with 10 similar floats that had been cleaned in the same manner was installed upstream of the pens a short distance in a direction that the tide never flows.

We were not able to clean anchor line in situ as the power washer can damage the fibers, so in instead we placed new anchor lines near the ends of the fish farm and on the reference float.

After commencing this work in spring of 2004, quarterly photographs of all sides of the floats and at three depths for the lines and nets were taken with an underwater camera. The camera was fitted with a distance measuring rod to maintain a constant distance to the substrate, although it was sometimes difficult to maintain that distance exactly due to strong currents. More than once we became entangled in netting during these dives, which is most disconcerting. At the completion of this work in late spring 2006 the plan was to recover all the substrate and take subsamples for quantitative counts and weights, as described above for the standing stock characterization.

Analyses Methods

Stable isotope analysis methodology

Samples were shipped on blue ice to the University of Idaho Stable Isotope Laboratory. http://www.cnrhome.uidaho.edu/isil . Upon arrival at the laboratory, samples were preprocessed by homogenizing the tissue in a mortar and pestle in liquid nitrogen before analysis.

An elemental analyzer (NC2500, CE Instruments, Milan, Italy) is used to liberate $N_2(g)$ and $CO_2(g)$ from solid samples by flash combustion, and subsequent oxidation and reduction reactions. A gas chromatographic column in the EA separates the two gas species which are vented to a mass spectrometer (Delta+, ThermoElectron Corp., Bremen, Germany) via a continuous flow interface (ConFlo II, ThermoElectron Corp., Bremen, Germany) for isotope ratio analysis (Figure 9). Standardized acetanilide are analyzed every 11 samples for assurance of stability, drift correction, and elemental mass fractions.

Normalization

Acetanilide working standards were calibrated against an acetanilide primary standard and are reported as a relative ratio to Peedee belemnite (PDB) for carbon and as a relative ratio to atmospheric nitrogen for nitrogen. The precision of this particular analysis was calculated from the standard deviation of the four secondary standard replicates. One standard deviation from the mean value was determined to be 0.12‰ and 0.14 ‰ for nitrogen and carbon dioxide, respectively. The drift correction applied to this analysis was –0.04 ‰ and –1.46 ‰ for nitrogen and carbon dioxide, respectively, based on the difference between the mean measured value and the known value of the secondary acetanilide standard.

Elemental Mass Fraction

The mean of the four acetanilide working standards were used to do a 1-point correction estimate of %C and %N.

Quality Control

A tertiary standard was used to verify the quality of the normalization applied. After normalization, this standard was determined to have δ^{15}N, [N], δ^{13}C, and [C] of 2.27 ‰, 2.82 %, -27.89 ‰, and 45.86 %, respectively. These values drifted from the know values by 0.01 ‰, 0.15 %, 0.39 ‰, and 1.64 %, respectively.

Figure 9. Stable isotope analysis equipment at the University of Idaho laboratory.

As studies of water use efficiency have increased, stable isotope analyses have become more critical and widespread within forest ecosystems and other biogeochemical systems research. The availability of these advanced "on-line" technologies greatly shortens analysis time and costs over traditional duel-inlet/vacuum-line work, which utilize techniques where the combustion, trapping and purification must be done manually for each individual sample prior to isotope analysis. The laboratory's instrumental precision and international referencing accuracy meets current industry standards

5. Results of Standing Stock Assessment

Types and quantities of submerged substrate

Existing substrate surfaces of floats, anchor lines and nets at the Site 3, Deepwater Bay fish farm near Cypress Island were analyzed to estimate the standing stock of invertebrates and algae. Submerged area estimates were applied to the results to estimate total wet and dry weight of the organisms by taxa and cumulatively for the entire farm. There are billions of invertebrates and algae associated with each farm and with limited research funds it was necessary to stratify the sampling to focus on key components and timings, as discussed below. Given the small sample size of the population, all of the estimates are approximations at best. No true replication of samples was possible due to time constraints involving the huge numbers of individuals often counted and identified in a single small area sample. There was large variation from sample to sample in some substrates such as floats, but in other types of samples such as netting the invertebrates were apparently more evenly distributed.

Net pen netting constituted the bulk of the submerged area substrate by an order of magnitude (18,432 m^2) or more for floats (1,020 m^2) and lines (122 m^2)separately (Fig. 10). The calculations only included one side of the netting so compared to lines and floats; the area was indeed large, equivalent to 90.5% of the total versus 0.6% for lines and 8.9% for floats. During 2004 no predator exclusion netting was present, but many net pens in Puget Sound use such large mesh netting to surround the entire pen assembly to exclude seals or sea lions from charging the nets. The nets are sized so as not to harm other species such as wild fish or marine birds that can and regularly do swim through the mesh to obtain biofouling organism on the net pen systems for feed.

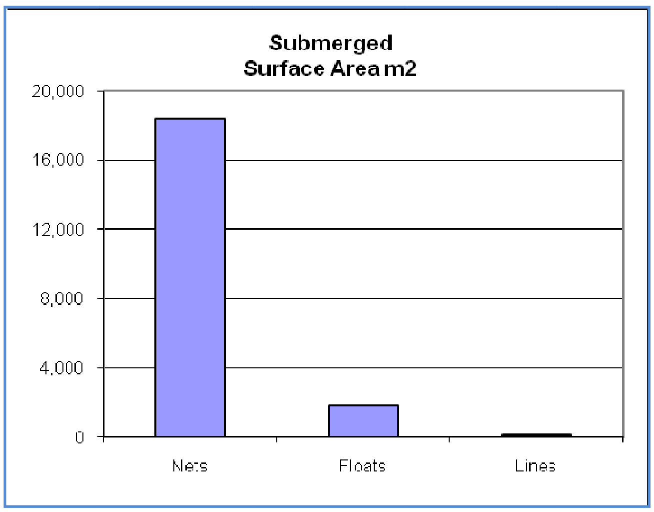


Figure 10. Submerged substrate area estimates (one side of nets only).

Basic statistics of sampling in Table 1 show that approximately 1/3 of the 99 net-pen colonizing species identified were plants and ~ 2/3 were invertebrates plus three species of fish. We collected, identified, enumerated and weighed over approximately 360,000 individual invertebrates in the standing stock assessment. Thousands of other individual samples were included in the separate colonization study discussed later in this report. Seaweeds were not enumerated because in some cases it was impossible to determine what constituted an individual. Accordingly, they were separated into specific taxa and weighed. Some minor amount of substrate was not sampled including the lines that are used for net weights and the weights themselves (steel pipe and cement weights).

Sampling area on floats averaged 710 cm^2. For nets it averaged 430 cm^2, 410 cm^2 and 680 cm^2 in winter, spring and summer, respectively. Anchor lines sampling area averaged 590 cm^2.

Overview of Species Assemblage

Table 1 summarizes some of the sampling statistics of the standing stock assessment. Nearly 360,000 individual invertebrates and fish were identified, enumerated, and weighed (wet and dry weights) for this part of the survey. Well over 100 species of invertebrates and fish were accounted for in this manner. Because of the extremely large numbers, it was impossible and impractical to identify some to genus level (e.g., polychaetes), although this was done when the differences were obvious (e.g., tube worms). However great care was taken to identify some species such as tunicates, to be sure we would identify any exotic (non-native) species that might have occurred.

Table 1. Standing stock assessment: basic statistics.

Total number of taxa (conservative estimate of species number)	99 (>100 including benthic diatoms or including all species)
Number of plants species	29
Number of invertebrate species	> 67
Vertebrate species (fish)	3
Number of individuals invertebrates and fish identified, enumerated and weighed (algae not included)	359,651
Sampling period seasons	Summer 2004: floats, nets, lines Winter 2004-05: nets Spring 2005: nets

The effect of depth on biocolonization of the nets was assessed in April and June 2005. This was done both to determine the effect and to correct biomass estimates that were normally made from the 1 m depth samples that could be collected by manually pulling up the net above the water line to sample. Depth was not a major influence on invertebrate biomass or species composition, but was a major influence on plant biomass. Replicate samples were taken from 1 m, 5 m and 10 m depth and processed as described in the method sections and comparisons made in total wet and dry biomass as well as species composition. Estimates were made with and without a dominant spring time invertebrate (*Ectopleura marina,* a hydroid). The analysis indicated that the 1m depth samples underestimated the total wet weight biomass by 35% in total. Variation of bay mussels accounted for the majority of the variance, and this variance was seen at other times of year too. Ectopleura marina biomass was 26% greater at mid depth (5 m) but the difference was due to weight of individuals, not abundance and was judged to be a product of random variation. Algal biomass was essentially zero at 10 m depth on the pens and very minimal at 5 m so only the 1 m depth measurements were conservatively applied to a 0 to 3 m depth range for calculating seaweed and algae biomass.

Species list

Table 2 presents a list of major taxa and species of colonizing organisms observed on net pen structures during this study.

Table 2. Fish and invertebrates observed on net pen floats, anchor lines and netting.

Functional grouping codes are 1: water filtering, 2: detritus feeder, 3: predatory, 4: herbivorous grazer, 5: mix of predacious and grazer, 6: autotrophic (photosynthetic).

Major Taxa or Phylum	Class	Genus species	Common Name or notes	Functional Grouping
Chordata	Actinopterygii	*Gobiesox maeandricus*	northern clingfish	3
Chordata	Elasmobranchii	*Raja rhina*	Longnose Skate	3
Chordata	Chondrichthyes	*Squalus acanthias*	Spiny Dogfish	3
Urochordata	Ascidiacea	*Chelyosoma productum*	Plate Head ascidian	1
Urochordata	Ascidiacea	*Distaplia occidentalis*	Red/purple "mushroom" ascidian	1
Urochordata	Ascidiacea	*Styela gibbsii*	brown, with red siphons ascidian	1
Urochordata	Ascidiacea	*Boltenia villosa*	solitary ascidian, hairy	1
Urochordata	Ascidiacea	*Pyura haustor*	Warty Sea Squirt (Stalk ascidian)	1
Urochordata	Ascidiacea	*Corella willmeriana*	Transparent Sea Squirt	1
Annelida	Polychaeta	*Sabellids Tubicolous Polychaetes*	Mostly sabellids	1
Annelida	Polychaeta	*Polychaete Errantia*	mostly nereids	3
Annelida	Polychaeta	*Halosynda brevietosa*	scale worms	3
Annelida	Polychaeta	*Polychaete sedentaria*	either	1
Annelida	Polychaeta	*Spirorbis sp.*	encrusting worm	1
Platyhelminthes	Turbellaria	*Notoplana acticola*	brown flat worm	5
Nemertea	Anopla	*Nemerteans*	ribbon worms	3
Cnidaria	Hydroza	*Ectopleura (Tubularia) marina*	Pink-top or pink mouth hydroid	1
Cnidaria	Hydroza	*Obelia dichotoma*	Branching hydroid	1
Cnidaria	Anthozoa	*Metridium senile*	common anemone	1

Major Taxa or Phylum	Class	Genus species	Common Name or notes	Functional Grouping
Cnidaria	Anthozoa	*Urticina crassicornis*	Green and red anemone	1
Cnidaria	Anthozoa	*Urticina cornea*	Strawberry red anemone	1
Cnidaria	Hydroida	*Plumulerin setacea*	hydroid	1
Cnidaria	Hydroida	*Abientinaria sp.*	hydroid	1
Arthropoda	Amphipoda	*Jassa marmorata*	tube amphipod	5
Arthropoda	Amphipoda	*Perampithoe sp.*	kelp borer	4
Arthropoda	Malacostraca	*Petrolisthes eriomerus*	Porcelain Crab	3
Arthropoda	Malacostraca	*Idotea wosnesenskii*	Isopod	3
Arthropoda	Maxillopoda	*Balanus glandula*	acorn barnacle 1	1
Arthropoda	Maxillopoda	*Semibalanus balanoides*	acorn barnacle 2	1
Arthropoda	Maxillopoda	*Balanus spp.*	mixed species of *Balanus*	1
Arthropoda	Malacostraca	*Cancer oregonensis*	Oregon cancer crab	5
Arthropoda	Malacostraca	*Pugettia producta*	kelp crab	5
Arthropoda	Decapoda	*Heptacarpus sp.*	broken back shrimp	3
Arthropoda	Pycnogonida	*Pycnogonid possibly Nymphon sp.*	Spider-like crustacean	4
Arthropoda	Amphipoda	*Caprella spp.*	Caprellids	5
Arthropoda	Malacostraca	*Oregonia gracilis*	decorator crab	5
Arthropoda	Malacostraca	*Mimulus foliates*	foliate kelp crab	5
Arthropoda	Malacostraca	*unknown*	crab zooae	1
Arthropoda	Maxillopoda	*Balanus nubilus*	giant barnacle	1
Arthropoda	Insecta	marine chironomid	true fly larvae, order Diptera, Clunioninae family	
Bryozoa	Gymnolaemata	*Membranipora membranacea*	Kelp encrusting bryozoan	1
Bryozoa	Gymnolaemata	*Dendrobeania lichenoides*	foliose "lichen" bryozoan	1
Bryozoa	Gymnolaemata	unknown spp.	bryozoans, foliose	1
Echinodermata	Asteroidea	*Pisaster ochraceus*	Starfish	4
Echinodermata	Holothuroidea	*Eupentacta quinquesemita*	pale orange cucumber	1 and 2
Echinodermata	Echinoidea	*Strongylocentrotus droebachiensis*	green sea urchin	4
Echinodermata	Holothuroidea	*Cucumaria miniata*	orange sea cucumber	1 and 2

Major Taxa or Phylum	Class	Genus species	Common Name or notes	Functional Grouping
Echinodermata	Ophiuroidea	*Ophiopholis aculeata*	brittle star (red body center)	2
Echinodermata	Holothuroidea	*Cucumatia lubrica*	sea cucumber (darker, usually deeper)	1 and 2
Mollusca	Bivalvia	*Mytilus edulis*	Edible common mussel	1
Mollusca	Bivalvia	*Chlamys hastata*	swimming scallop (small)	1
Mollusca	Bivalvia	*Cassostrea gigas / Ostrea conchaphila*	Japanese or native oysters	1
Mollusca	Bivalvia	*Pododesmus cepio*	Jingle shell/Rock Jingle	1
Mollusca	Polyplacophora	*Mopalia sp.*	unknown chiton	4
Mollusca	Gastropoda	*Aeolidia papillosa*	"Shaggy mouse" nudibranch	3
Mollusca	Gastropoda	*Hermissenda (Phidiana) crassicornis*	"fluorescent" nudibranch, eggs common too.	3
Mollusca	Gastropoda	*Dirona auranitia*	Orange nudibranch, white tips	3
Mollusca	Gastropoda	*Aeolidia papilosa*	"shaggy little mouse" nudibranch	3
Mollusca	Bivalvia	*Hiatella arctica*	nesting or rock dwelling clam type	1
Mollusca	Gastropoda	*Littorina sp.*	Periwinkle	4
Mollusca	Bivalvia	*Modiolis rectus*	hairy clam	1
Mollusca	Polyplacophora	*Cryptochiton stelleri*	gumboot chiton	4
Mollusca	Bivalvia	*Crassostrea gigas*	Pacific oyster	1
Mollusca	Gastropoda	*Tectura scutum*	Pacific plate limpet	4
Mollusca	Gastropoda	*Diodora aspera*	keyhole limpet	4
Mollusca	Gastropoda	*Collisella pelta*	variable limpet	4
Mollusca	Gastropoda	limpet spp.	too small to ID or other	4
Mollusca	Gastropoda	snail, unknown sp.	unknown	4
Porifera	Calcarea	*Scypha spp.*	Small white vase sponge	1
Porifera	Calcarea	*Halichondria bowerbanki*	often associated with tube worms	1

Table 3. Algae observed on net pen floats, anchor lines and netting.

Functional grouping codes are 1: water filtering, 2: detritus feeder, 3: predatory, 4: herbivorous grazer, 5: mix of predacious and grazer, 6: autotrophic (photosynthetic).

Major Taxa or Phylum	Class	Species	Common Name	Functional Grouping
Chlorophyta	Ulvales	*Ulva spp.*	Ulva and Enteromorpha combined	6
Chlorophyta		*Enteromorpha intestinalis*	tube form	6
Chlorophyta		*Enteromorpha linza*	Tube-to-blade	6
Chlorophyta		*Lola lubrica*	(common near Fraser River delta)	6
Chlorophyta		*Codium fragile*	dead man's fingers	6
Chlorophyta		*Codium setchellii*	no common name	6
Chlorophyta		green algae spp.	Unidentified, on nets mostly	6
Phaeophyta		*Fucus gardneri*	Rockweed	6
Phaeophyta		*Nereocystis luetkeana*	Bullwhip kelp	6
Phaeophyta		*Laminara saccherina*	No mid-rib blade	6
Phaeophyta		*Alaria marginata*	Ribbon or Winged algae (mid-rib blade)	6
Phaeophyta		*Pterygophora californica*	Stalked kelp	6
Phaeophyta		*Costaria costata*	five-ribbed kelp	6
Phaeophyta		*Desmarestia ligulata*	acid seaweed	6
Phaeophyta		*Cystoseira geminate*	northern bladder chain kelp	6
Phaeophyta		*Sargassum muticum*	wire or strangle weed (exotic, Japan)	6
Various		diatoms, benthic or facultative	"brown algae" on nets, Melosira sp.	6
Phaeophyta		*Desmarestia aculeate*	witch's hair	6
Rhodophyta	Bangiophyceae	*Porphyra sp.*	Thin red algae	6
Rhodophyta	Floridephaceae	*Callophyllis edentate*	Pink, veined red algae	6
Rhodophyta	Floridephyceae	*Delesseria decipiens*	Mid-rib red algae	6
Rhodophyta	Rhodophyceae	*Chondracanthus exasperatus*	Turkish towel (previously Gigartina)	6
Rhodophyta	Rhodophyceae	*Membranoptera platyphylla*	red algae	6
Rhodophyta	Rhodophyceae	*Palmaria hecatensis*	edible dulse-like species	6

Major Taxa or Phylum	Class	Species	Common Name	Functional Grouping
Rhodophyta	Rhodophyceae	Unknown, possibly several species	red algae, filamentous spp.	6
Rhodophyta	Rhodophyceae	Unknown, possibly several species	red algae, finely branched	6
Rhodophyta	Rhodophyceae	Unknown	red algae, foliose	6
Rhodophyta	Rhodophyceae	Unknown	red algae, encrusting	6
Anthophyta	Liliopsida	*Zostera marina*	eel grass (not rooted but trapped)	6
Not applicable	Not applicable	Not applicable	Detritus, unidentified matter	NA

Biomass estimates

Table 4 and Figures 11 to 13 summarize overall results of biomass estimates of standing stock. Intense sampling was conducted in the summer of 2004 on all substrates and the subsequent winter and spring on netting.

Table 4. Summary of biocolonization biomass (wet weight, metric ton) estimates for a single farm site's nets, lines and floats from the standing stock data.

Substrate	Season	Mean Weight Tons (wet)	Standard Error	N Discrete Samples	N Invertebrates Analyzed *
Floats	Summer	15.9	3.1	26	30,347
Lines	Summer	23.8	7.1	14	270,303
Nets	Spring	24.6	7.4	10	7,203
Nets	Summer	15.9	4.6	16	54,789
Nets	Winter	4.5	1.39	16	23,710
All surfaces	Summer	55.6	14.9	56	386,352

* Number of organisms identified, enumerated and weighed includes invertebrates only as algae could not be reliably counted and attributed to a single plant. Variability estimates are given in subsequent tables.

From this table and associated figures it is seen that:

- Total summer biomass of net, float and line biofouling was estimated to be 55.6 metric tons, wet weigh (95% confidence interval = 45.6 to 65.5 MT). This is a large amount of material, but is only about 5% of peak farmed fish biomass held at the site during this study.
- Surprisingly, the biomass of biocolonization of lines exceeded that of nets or floats in summer. The volume of colonization on lines was particularly surprising given the much smaller surface area (0.6% of total). Lines are particularly good substrate for seaweed colonization of bull kelp, which constituted about 2/3 of the total wet weight during summer.
- Much higher rates of biocolonization were recorded on nets in the spring (+61%) while winter values declined markedly (-71%) compared to summer. This is both a function of recruitment and net cleaning practices.
- Surprisingly, the biomass of caprellid (skeleton) shrimp and amphipods remained high throughout the year. Winter population reproduction was likely less than summer and standing stock is not a good measure of juvenile recruitment because net washing does not occur in the winter. So despite probable reduced reproduction and recruitment rates, the standing stock of these species was comparable to the warmer spring and summer seasons.

- Algae (seaweed and attached diatoms) biomass was minimal on the nets compared to invertebrates during all sampled seasons. This was expected as the nets are maintained to prevent algal fouling (by initial copper treatment and subsequent power spraying).
- Anchor lines proportionally and nominally had the most algal materials, followed by floats, corresponding to the degree of exposure to sunlight. Much of the float surfaces are shaded by the overlying walkway or are on the bottom (downward-facing) surface where sunlight is attenuated. Anchor lines are more exposed to sunlight and at depth become chains, which are not generally colonized by algae or invertebrates.

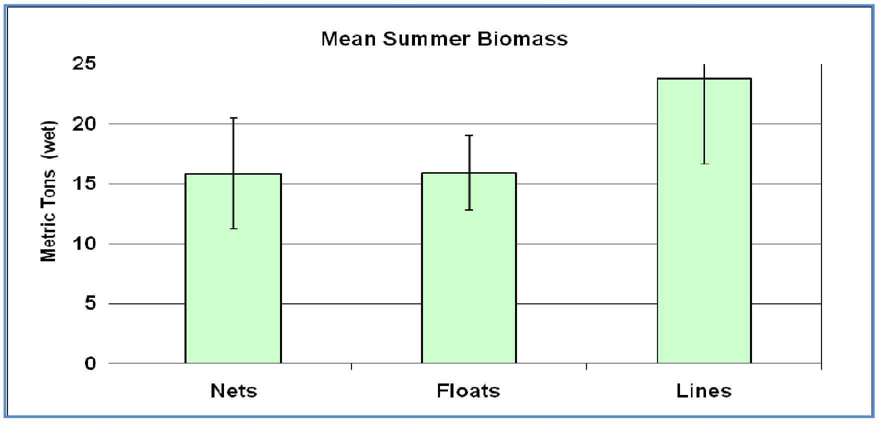
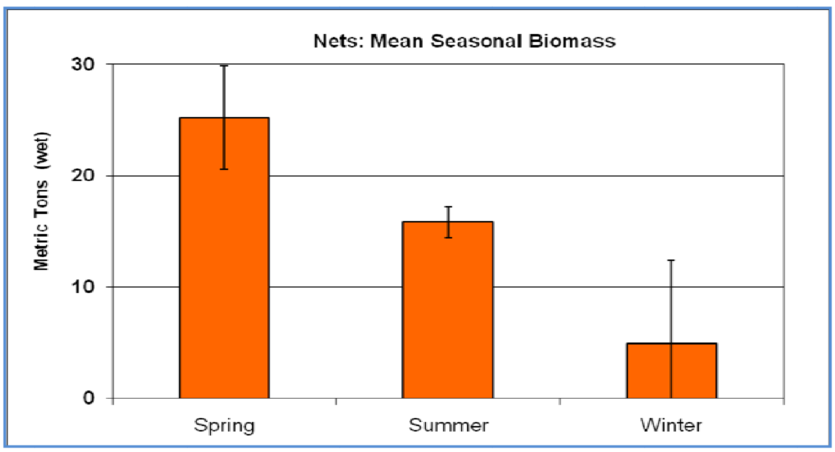


Figure 11. Mean summer biomass wet weight for nets, floats and lines with SE bars.

Figure 12. Mean seasonal biomass for netting surfaces with SE bars.



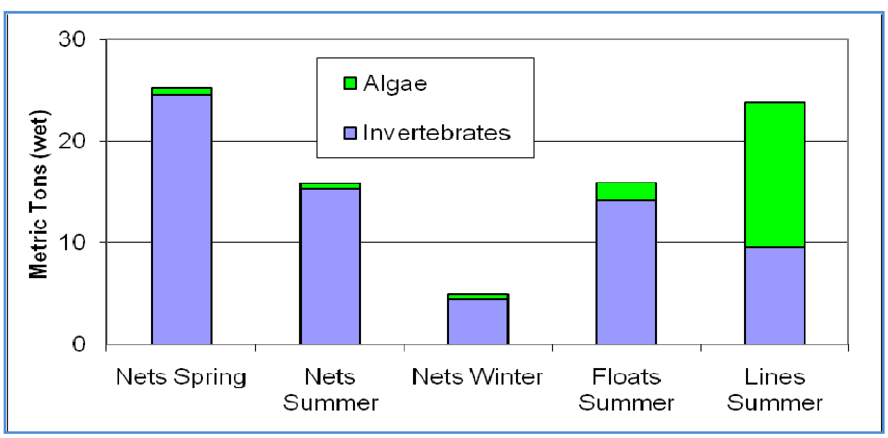


Figure 13. Mean seasonal biomass for algae versus invertebrates on differing substrates.

Standing Stock: Dominant species analyses

This section presents the results of species biomass estimates by season and species. We begin with floats, then discuss nets and finally anchor lines.

Floats

During summer, the dominant species by weight occurring on the floats were barnacles, mussels, brown seaweeds (*Costaria costata & Alaria marginata*) and others shown in Table 5. The wet weight of seaweeds would have been much greater for the early summer alone, but the data summary includes the whole summer through September and seaweed biomass peaks in late spring and early summer at this site.

Abundance rankings of invertebrates were led by barnacles, caprellid shrimp, amphipods, polychaete worms, sea squirts (*Corella willmeriana*) and small crabs, mostly the "Oregon" or pigmy crab (*cancer oregonensis*). The latter commonly feeds on barnacles and the pelagic larvae are abundant in the bay in spring and early summer.

It is not surprising the barnacles were abundant as they are often dominant in the near surface zone of all hard substrate and splash zone of Northeast Pacific waters. In terms of area occupied, they did not seem to be overly dominant on the floats, but with their calcareous shell their weight makes them dominant in terms of total biomass. Should we have measured only live, fleshy tissue of barnacles, a near impossible task given sampling logistics, they would have ranked much lower in the biomass ratings. Barnacles of the genus *Balanus* have been found to accelerate settlement and growth of seaweed because grazers such as limpets (i.e., gastropods with hat-like shells) are unable to utilize the rough surface created by barnacle "tests" (i.e., term for barnacle shells, see Farrell 1991 for more on limpet/barnacle/algal interactions).

Mussels were approximately as common as barnacles in terms of occurrence or non occurrence (Table 5) but their cumulative wet or dry weight was approximately 3 to 4 times less than barnacles with their relatively heavy, calcareous shells.

Nets

Nets were sampled in spring, summer and winter with a ranking summary of biomass in wet weight shown in Table 5 and simplified ranking of dominant species only in Table 6. Considerable variation among seasons was noted as discussed below. Later in this report we describe facets of the natural history of the principal species.

In the summer and winter caprellid shrimp were the dominant species by weight which is remarkable given the small size of the individual "shrimp" which are actually amphipods (as discussed later). They were in 88% of the samples and averaged 383 g m^{-2} on the netting in summer (Table 6).

In spring the pink top hydroid (*Ectopluera marina*) was the single most prolific organism. The growth of these hydroids is spectacularly fast, but equally as fast is their decline. The distal "flower" ends appear to be grazed rapidly but the grazer was not observed, as discussed later.

Table 5. Wet and dry weight areal estimates for differing substrates at the subject fish farm.

	Wet Weight Ranking, Decreasing	Frequency of Occurrence (in sample or not)	Mean wet weight per sample g/m2	Mean dry weight per sample g/m2	Abundance Ranking (NA for Algae)	Mean no. per sample when present	SD No. per sample when present
Floats Summer							
Balanus spp.	1	96.2%	3,111.1	1,800.9	1	357.0	792.6
Mytilus edulis	2	96.2%	1,387.3	486.6	7	9.9	12.2
Costaria costata	3	11.5%	1,346.4	159.5	--	--	--
Metridium senile	4	65.4%	1,174.8	178.9	9	8.9	14.8
Alaria marginata	5	15.4%	1,088.1	166.3	--		
Urticina crassicornis	6	46.2%	443.7	81.0	11	3.0	2.1
Detritus	7	84.6%	374.8	72.6	--	--	--
Nereocystis luetkeana	8	7.7%	294.2	23.4	--	--	--
Corella willmeriana	9	15.4%	213.4	17.0	5	43.0	60.2
Fucus gardneri	10	7.7%	182.0	27.2	--	--	--
caprellid shrimp	11	92.3%	173.8	30.2	2	352.9	597.2
polychaete worms	12	96.2%	137.3	25.6	4	75.5	242.0
Ulva spp.	13	53.8%	106.7	8.9	--	--	--
Jassa spp.	14	100.0%	70.8	16.1	3	263.6	530.6
Chelyosoma productum	15	11.5%	56.7	4.7	13	1.3	0.6
Cancer oregonensis	16	23.1%	50.8	15.0	6	11.7	15.1
Distaplia occidentalis	17	23.1%	15.7	1.4	10	8.8	11.0
Perampithoe sp.	18	34.6%	9.9	1.5	8	9.2	7.4
Ectopluera marina	19	7.7%	9.1	1.4	12	3.0	2.8
Nets Summer							
Caprellid shrimp	1	87.5%	383.0	70.4	2	1,363.7	2,383.6
Filamentous diatoms & algae	2	62.5%	340.5	47.2	--	--	--
Jassa spp. amphipods	3	93.8%	169.0	33.4	1	1,857.1	3,646.7
Ulva spp.	4	56.3%	105.2	11.0	--	--	--
Mytilus edulis	5	25.0%	25.9	10.3	3	4.5	6
Polychaete worms	6	12.5%	2.2	0.4	4	2.5	2.1
Nets winter							
Caprellid shrimp	1	56.3%	2,946.7	509.5	2	512.0	1619.8
filamentous diatoms & algae	2	106.3%	100.5	23.0	--	--	--
Jassa spp. amphipods	3	112.5%	74.7	18.3	1	870.7	1159.3
Alaria marginata	4	100.0%	72.9	10.77	--	--	--
red algae	5	25.0%	40.7	8.2	--	--	--
Polychaetes	6	75.0%	31.7	8.6	3	50.0	99.5
Ulva spp.	7	50.0%	24.6	4.8	--	--	--
Mytilus edulis	8	18.8%	22.8	9.0	4	12.3	22.5
Nets spring							

	Wet Weight Ranking, Decreasing	Frequency of Occurrence (in sample or not)	Mean wet weight per sample g/m2	Mean dry weight per sample g/m2	Abundance Ranking (NA for Algae)	Mean no. per sample when present	SD No. per sample when present
Ectopluera marina	1	22.2%	781.36	144.6	3	64.8	61.8
Mytilus edulis	2	77.8%	331.4	111.2	4	9.7	15.2
Jassa spp. amphipods	3	100.0%	314.8	71.7	1	489.0	356.6
filamentous diatoms	4	100.0%	222.7	48.6	--	--	--
Caprella spp.	5	77.8%	159.4	31.9	2	210.7	353.1
Costaria costata	6	22.2%	48.2	5.5	--	--	--
red algae	7	77.8%	21.3	3.1	--	--	--
Polychaete worms	8	33.3%	12.7	3.5	5	5.5	5.0
Lines summer							
Alaria marginata	1	14.3%	197,524	29,610.5	--	--	--
Nereocystis luetkeana	2	100.0%	65,627.6	6,172.7	--	--	--
polychaete worms	3	100.0%	39,485	9,987.4	3	49.7	60.5
Caprella spp.	4	100.0%	11,698	2,328.4	1	12,10	41697
Laminaria saccherina	5	28.6%	5,732	739.2	--	--	--
Mytilus edulis	6	92.9%	5,458	1,940.2	7	14.8	13.2
Ulva spp.	7	64.3%	2,234	393.0	--	--	--
Chelyosoma productum	8	42.9%	1,925	182.8	6	16.3	23.1
Eupentacta quinquesemita	9	78.6%	1,825	507.4	8	14.8	25.9
Metridium senile	10	57.1%	1,402	271.6	11	5.8	4.9
Red algae	11	64.3%	1,369	213.0	--	--	--
Perampithoe sp.	12	50.0%	854	154.4	5	18.3	16.9
Pyura haustor	13	14.3%	692	113.2	18	2.0	0.0
Jassa spp.	14	100.0%	578	139.1	2	5,380.0	14440
Cancer oregonensis	15	21.4%	489	153.9	4	19.9	13.3
Pugettia producta	16	35.7%	351	125.8	15	3.2	3.3
Styela gibbsii	17	78.6%	312	45.8	9	8.7	6.6
Urticina spp.	18	28.6%	290	56.6	17	2.2	1.1
Detritus	19	100.0%	241	32.2	--	--	--
Modiolis rectus	20	50.0%	205	89.1	16	3.0	2.8
Boltenia villosa	21	28.6%	129	17.8	10	6.0	1.0
Obelia dichotoma	22	57.1%	49	9.2	20	1.4	0.5
Cucumaria spp	23	42.9%	32	8.1	19	1.8	47.8
Balanus spp	24	64.3%	28	15.4	14	4.3	3.4
Hiatella arctica	25	85.7%	18	9.2	12	5.2	4.4
Nemertians	26	42.9%	16	3.3	13	5.0	4.34
Notoplana acticola	27	21.4%	3.9	1.2	21	1.3	0.58

* Nereocystis were inventoried separately from every anchor line as explained in the methods.

Consistently ranking third in each season was the tube-dwelling amphipod (*Jassa* spp.), a species that, despite their tiny size compared to most other biocolonizing macroinvertebrates, was extremely numerous. This genus included both introduced species and native species as discussed below.

Mussels (*Mytilus edulis*) were present in all sampling periods but were more prolific in the spring. Sets of larval mussels of course occur in the spring but net cleaning and predation by sea birds reduces their abundance. Without these sources of loss, especially the sea birds discussed later, the nets could easily become heavily loaded and unmanageable as was seen in some early trials of net pens in southern Puget Sound in the 1970s (Snyder et al. 1976).

Table 6. Summary of standing stock biomass ranking (wet weight) for nets in three seasons.

Taxa	Spring	Summer	Winter
Caprellid shrimp	5	1	1
Pink-top hydroid	1	0	0
Filamentous diatoms	4	2	3
Amphipods (*Jassa* spp.)	3	3	3
Costaria costata (brown seaweed)	6	-	-
Bay mussel (*Mytilus edulis*)	2	5	8
Alaria marginata ("ribbon kelp")	-	-	4
Red seaweeds	-	-	5
Ulva (green seaweed "sea lettuce")	-	5	7
Polychaete worms	8	6	6

Polychaete worms ("errantia" or tubeless, mobile worms) were also a year round resident of the nets, in many cases subsiding partly or totally within the weave of the knotless netting. The sessile, tube-dwelling ("sedentaira") polychaete worms of the family Sabellidae seen on the anchor lines were uncommon on the nets.

Filamentous, benthic or facultative (capable of growing on surfaces or in pelagic, free-floating form) were present year round with significant growth mostly in the summer. These diatoms are both a source of grazing fodder for some invertebrates and an attachment point for larval or juvenile invertebrates seeking substrate to settle upon.

Seaweed also displayed some interesting variability. Many of these species begin growth very early on in the year in winter, such as *Alaria marginata*, a brown algae commonly identified by its prominent stripe that runs longitudinally down the center of its blade. A variety of red seaweeds a class that includes some high commercial value species also began to become prominent in late winter. *Costaria costata* (five-ribbed kelp) biomass peak was noted later, in the spring season. None of the seaweeds were prevalent in summer on nets with the exception of *Ulva* ("sea lettuce") that occurred with spotty distribution on the shallow fringe of some of the nets.

Figure 14. Atlantic salmon subadult fish inspecting our net patch job after a section of netting was removed for species enumeration and identification.

Net cleaning by farm staff commences in spring and involves pulling of the nets to the surface, air drying (in some cases), removal and shore disposal of large clusters of colonization and power washing *in situ*. Cleaning is necessary as a best management practice to avoid clogging of the nets and the heavy weight of biocolonization that could endanger the structural soundness of the nets. Cleaning in spring and summer of course affected the succession that would otherwise occur on the nets, but the purpose here was to quantify what exists at net pen sites, not what would occur without any human perturbations.

Some may argue that net cleaning in situ is an unsound environmental practice. With regard to the organic matter released, it does behoove the growers to minimize the amount of organic matter but these discharges are not unaccounted for in the regulatory process. Net pens are required to meet stringent performance requirements in terms of distance of measurable effect on the seabottom. The organic matter released from net cleaning contributes to the benthic loading not unlike waste fish feces or waste feed. If net cleaning was a significant adverse effect it would be detected in the monitoring process. In terms of the copper released from the pens during cleaning, monitoring of the status of the sea bottom is also required and to date has shown results similar to background conditions in all cases. Nevertheless, the paint industry is working toward the goal of providing non-toxic antifoulant paints with some trials presently underway in Chile and elsewhere (Pers. Comm. Steven W. Fisher, Global Director, Marine Coatings, PPG Industries).

Lines

Although anchor lines accounted for only a small fraction of the total available submerged area, the density of algae and invertebrates far exceeded other substrate with a diverse assemblage of many species (Table 5). This means that biomass per unit substrate area was greatest with these lines. Had the sampling been earlier in the summer or in late spring the seaweed biomass would have been even greater as there were signs that the fronds were “past their prime” by the time sampling was conducted.

Alaria marginata, the economically important “ribbon kelp” was abundant on the anchor lines during summer sampling. The alga was not highly frequent in occurrence in each short piece of line sample, but that was an artifact of the sampling size and zonation near the surface. Its large size and weight of individual holdfasts and fronds caused its wet and dry weight to exceed other species (except bull kelp) by a factor of at least 5 for the second most prevalent species.

Sabellid ("feather-duster") polychaete worms were second in terms of weight contribution on anchor lines. These beautiful and large tube worms have large, leathery tubes of up to 20 cm (Kozloff 1983) and in this author's experience are relatively common in pristine, highly flushed channels and passages of Puget Sound such as on submerged rock faces in Deception Passage or similar habitats in the San Juan Islands and surrounding areas. They feed by using the colorful feeding cirri (i.e., tentacles) that are arrayed with small cilia to create water currents that send small food particles to collecting grooves and the mouth (Kozloff 1983). At the subject fish farm, these worms were generally deeper than the seaweed discussed above and represent the "climax community " of hard or semi-hard substrates. Their intertwined tubes create a productive matrix for other species to colonize which in large part accounts for the great biomass observed on these lines. Fish farmers periodically replace lines as part of best management practice maintenance schedules, but the worms are able to establish these dense colonies within a few years and recruitment of juveniles via pelagic distribution is apparently common each year. Missing from the lines are the coiled and white tube of serpulid worms (*Serpula vermicularis*) such as that often co-exist on rocks with sabellids worms. As with barnacles, the lines will bend or flex at times which prevents some hard surface colonizers from growing to any appreciable size.

Caprellids are next in order of biomass weight which was surprising to this author. Although very prevalent on the nets, their importance on anchor lines was due to their colonization of seaweed fronds. We spent long hours removing them individually from the fronds, even in death their grip on the algae remained tight in many cases. They were discussed previously in more detail.

The biomass dominant species of the net-pen floats, barnacles, were largely absent from the lines. This is likely due to the flexible nature of the lines and occasional bending that may occur as well as the slightly coarse and fibrous surface of the lines which does not simulate the hard surfaces such as that afforded by rocks. Species depth zoning also plays a role, barnacles prefer the high intertidal or immediate splash zone of marine habitats while the anchor lines span the entire depth range from surface to near bottom, where chain is typically used instead of line.

Dominant Species Analyses Functional Grouping Analysis: Diversity

To further characterize the biota of the submerged farms surfaces, the observed species were coded as to their likely biological mode of sustenance into 6 possible functional groups:

1) water filtering
2) detritus feeder
3) predatory
4) grazer (herbivore)
5) mixed predator and grazer
6) autotrophic (photosynthetic)

The occurrence of each species or taxa was then summed for each sample and the percent occurrence calculated in total. Note that this is not a measure of abundance or biomass! It is a kind of diversity measure only. It also includes organisms of different phyla within a category, for example sabellids polychaete worms and shellfish such as mussels along with crustaceans such as barnacles in the filtering category.

Most of the species were either filter feeders of a mix of predacious and herbivorous and are shown in Table 5 by percent frequency. The largest difference was between floats vs. lines and nets with the former having >50% as filtering organisms, but the latter less than 28% in all cases. For floats, this reflects the abundance of anemones, mussels, barnacles and other filtering organism versus the other substrates with their frequent occurrence of amphipods and other grazers or predators.

Table 5. Functional group frequency of occurrence of species among differing substrates at the subject fish farm. Line estimate does not include *Nereocystis luetkeana*.

	filtering	filter-detritus	predacious	herbivore	mix	algae
Floats	54.6%	1.1%	4.8%	7.0%	21.8%	10.7%
Lines	11.3%	--	3.2%	--	46.8%	38.7%
Nets, Spring	28.6%	--	12.5%	--	32.1%	26.8%
Nets, Summer	10.8%	--	3.1%	--	47.7%	38.5%
Nets, Winter	10.3%	--	10.3%	--	35.9%	43.5%

The dominant species of each substrate was discussed in detail previously. It is not surprising to see many of these results such as the dominance of filter feeders on floats. But for lines, the mixed predator/grazer category was more diverse than the seaweeds.

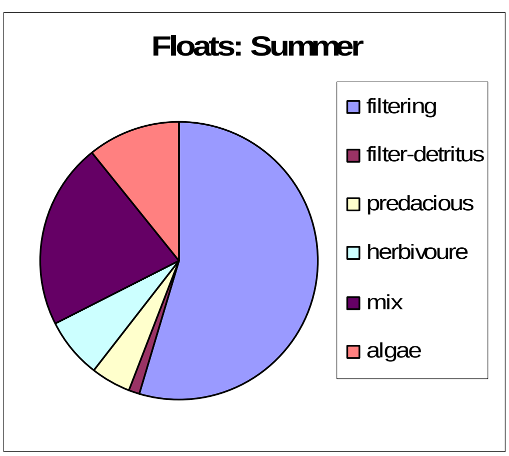
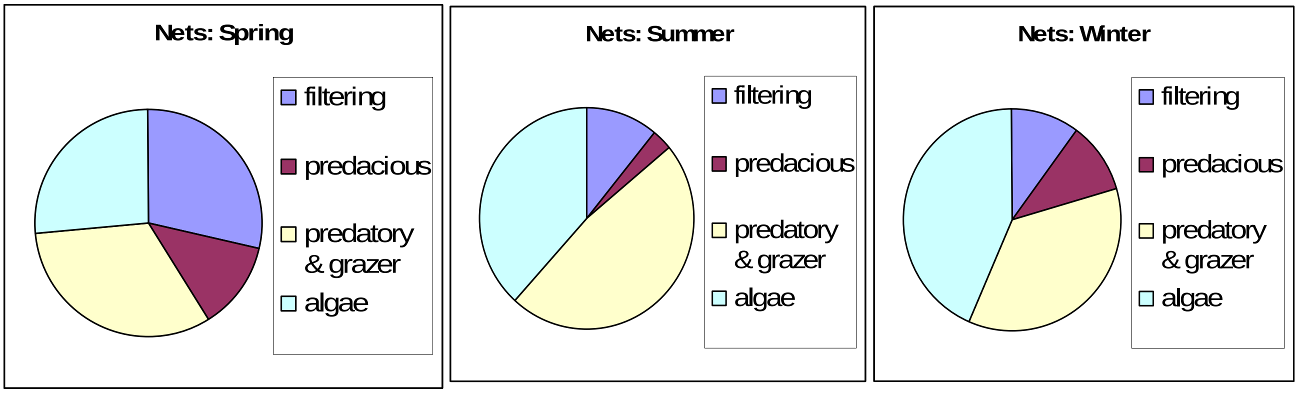


Figure 15 . Percent occurrence of functional groups of invertebrates and algae by species on floats during summer.

Figure 16 (below). Percent occurrence of functional groups of invertebrates and algae by species on nets during spring, summer and winter.



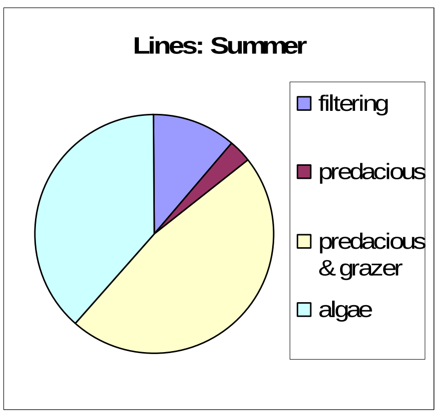


Figure 17. Percent occurrence of functional groups of algae and invertebrates by species on lines during summer season.

Dominant Species Characterization

Of the 100+ species that were identified, a few dominant species in terms of biomass or number are worth focusing special attention upon. Here we provide some background information on these species as well as a few that are simply interesting. There are few other quantitative assessments of the biota of human-constructed, submerged structures in Puget Sound, although the well known volume by Kozloff (1983) provides an indispensably-important reference as to what is seen in general for other types of structures including dock floats in the region.

Caprellids (skeleton shrimp)

Skeleton shrimp are amphipods of the order Caprellidea and highly abundant on net pen nets in Puget Sound year round (Fig. 18 and 19). Amphipods are generally small crustacean of the class Malacostraca (crabs, krill, pill bugs, shrimp, and relatives) in their own order, Amphipoda. Amphipods are considered a most efficient scavenger of sea bottoms and intertidal shores, likely clearing up and recycling more organic shore debris than any other animal (Schmitt 1968, Staude et al. 1977). There is little doubt that they constitute some of the highest concentrations of this species that occur anywhere in Puget Sound. As the name indicates, the thin, long body of these skeleton shrimp is adapted to allow them to blend into blades or filaments of seaweed, sponges, hydroids and bryozoans on which they are also found. They are not found on the sandy bottoms near the fish farms or in other sandy or muddy bottoms of Puget Sound without the substrates mentioned above.

There are many species of caprellids, no attempt was made to identify species here but most are predators, others are filter feeders. They have the ability to change colors relatively rapidly and for predator species that are ambush predators that allows them to wait motionless until prey comes by. Prey includes small worms and a variety of other invertebrates including amphipods. It is not known if they feed on *Jassa marmorata* that are so abundant on the nets too but this could be determined relatively easily. Kozloff (1983) and others suggest they feed on the polyps of hydroids, but others feed mostly on diatoms and detritus. They may prey on the solitary pink hydrozoan *Ectopluera* (*Tubularia*) *marina,* discussed below.

Other species of skeleton shrimp are detritus or filter feeders and according to the Monterey Bay Aquarium on-line field guide, they "play an important role in the ecosystem by eating up detritus and other food particles". The same source also states that they are consumed by sea anemones, surfperch and shrimp. Other marine fish are known to consume caprellids and so rearing of marine fish in cages may provide the fish with a supplement to their diet. Use of non-toxic net treatments to reduce net biocolonization would be desirable in that case. Prior work on the feeding habits of chinook salmon in net pens showed that caprellids or other forms of biocolonization are not commonly or even rarely consumed by the caged fish (Moring 1975, Rensel 1976).

Figure 18. Caprellid shrimp removed from netting (photo by Michael Womer).

Female skeleton shrimp kill the males after mating, using venom injected by a venomous claw. Egg clusters are often highly visible on the thorax of the females.

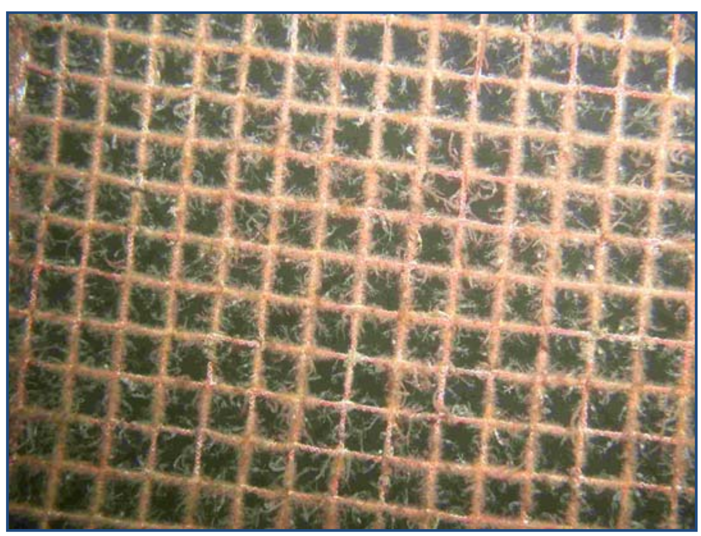


Figure 19. Underwater photograph of caprellid shrimp on netting. Jassa spp. amphipods are present in high numbers too, but too small to show in most photographs.

Amphipods

Two other genera of amphipods were observed in this survey, *Jassa* and *Perampithoe,* the former being extremely common and abundant on netting year around and tentatively including at least two species, *J. marmorata* and *J. staudei.* The former is a non-native species that is common along the entire North American west coast that was first observed there in 1941, apparently introduced from Great Britain (Fig. 20). There are no records of the first appearance in Puget Sound but for central Puget Sound it may have been after the mid 1970s, as the primary author conducted a net fouling study at that time at the EPA dock near Manchester and did not observe the species but dominants included mussels, ascidians, Spirorbis sp. and various polychaetes (Rensel and Prentice 1978). *Perampithoe* (kelp borer) is presumably a native species that was described from Barkley Sound and occurs throughout S. British Columbia. Identifications of all three species were provided by Jeffery Cordell, Principal Research Scientist, School of Aquatic and Fisheries Sciences, University of Washington, but he looked at only a small subsample and there may be other species present as dozens of species of *Jassa* are known to occur worldwide and many Jassa spp. are morphologically very similar.

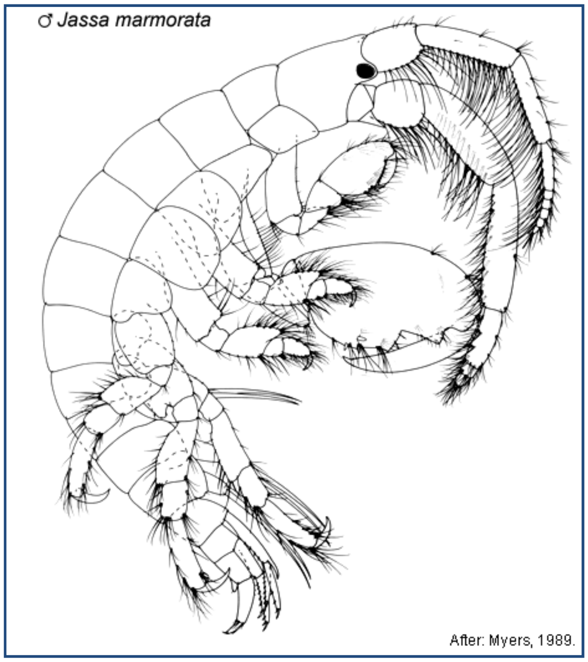


Figure 20. Line drawing of *Jassa mamorata* from Meyers (1989, permission requested).

Jassa forms tubes among algae, sponges, tunicates and on solid surfaces as a fouling organism (Myers 1989). We did not make detailed observations of the species morphology, but in all cases they were observed by us without tubes. There may have been small tubes inside the mesh of the netting, but these were not observed. It may be that very small juveniles are tube dwelling while the adults or adults males only are free ranging as the literature indicates sexual dimorphism and life stage changes in behavior. The diet of these amphipods at the net pens also remains uncertain. In other areas some maintain that they are filter feeders on diatoms and plankton, although sometimes cannibalistic or predatory on competing organisms (Prince William Sound Regional Citizens' Advisory Council 2004), but more likely the individuals we collected and observed were grazers on the benthic diatoms and hydroids that grow on the cages.

It was not uncommon to have thousands of these amphipods per sample, and up to several hundred thousand per square meter and a maximum of over 300,000 per m^2 calculated. Not one of these was seen within a tube but the tubes may be within the netting twine and very tiny. In England high densities have been observed in a seawater system inflow, and the authors also believe that this amphipod feeds outside its tube (Dixon and Moore 1997). These differences suggest that the local species is not *Jassa marmorata*, so more study is needed. Although an exotic species, they appear to be colonizing a niche not previously used by amphipods in Puget Sound. Many other species of gammaridean amphipods exist in Puget Sound, about 200 in one review (Staude et al. 1977). No comprehensive assessment is available, but at least some of the species are known to reproduce year round. This species has not been noticed in grab sampling of the bottom around pens, but NPDES sampling is scheduled in the summer of 2007 and special attention will be given to it. Tisch (1977) reports poor swimming ability for this species so survival may be limited after dislodgement from the pens, but these individuals may still provide fodder for downstream predators. The same author provides growth, survival and fertility estimates for east coast U.S. populations and reports several overlapping generations per year, which we suspect is true for Puget Sound too given the obvious biomodal or trimodal size distributions within the population we observed at any one time. We did not observe amphipods of the genus *Corophium* or their stout tubes, that are common in the Columbia River and also some parts of Puget Sound where siltation is heavy (Staude et al. 1977).

The *Jassa* spp. amphipods observed at the fish farm were unusual in one regard. As discussed above, they are reportedly tube dwellers but have adapted to a high energy environment where strong currents occur periodically that could dislodge them from the net pens if they didn't cling to the netting with tenacity. They may be dislodged, but the small bait fishes that regularly occur downstream of the cages may benefit from their removal. But this is only speculation; some stomach sampling might help verify this hypothesis. The small bait fish are usually present downstream where there is less current but also large particles of waste feces or occasionally feed that are emitted from the pens. The shedding of biocolonizing organisms is another form of wastes coming from the pens but in such physically active environments the food web is capable of assimilating the wastes in an aerobic manner, leading to no real biological loss and most likely a gain of diversity and abundance in such cases (Pearson and Rosenberg 1978).

Anemones

Anemones are perhaps the largest and most visible bio-colonizing invertebrate seen on the net pen floats. Most are the common white anemone, *Metridium senile*, but other species are present too as shown in Tables 5 and 6.   These invertebrates are very long lived, some known to have existed for over a century.

Figure 21. *Metridium senile* anemones on the surface of a net pen float (left side).

They reproduce both by sexual and asexual means; the latter method consisting of budding that spreads and become physically dominant on some surfaces of the floats and in some sections of anchor lines. Yet they never become entirely dominant on the floats, despite the appearance of suitable, un-colonized surfaces seen scattered randomly on unperturbed floats.   The anemones provide considerable filtering of the surface waters around the pens, but as discussed later, they apparently prefer live zooplankton. Anemones produce a surface mucus material and probably help trap floating materials on the net pen floats, such as errant eel grass commonly seen on these floats during the growing season (Fig. 21).

Ectopleura (Tubularia) marina (Pink-top hydroid)

The prolific hydroid *Ectopleura* (*Tubularia*) *marina* commonly called the pink-toped or pink mouthed hydroid occurs in mid spring on the netting for a short period in very dense numbers. It is first visible as a pinkish flower-like organism, but is rapidly grazed, probably by caprellid shrimp and other predators. On our untreated net panels we found a huge abundance of these organisms in April, but the commercial net pens are never allowed to foul to the point seen in Figure 22. This hydroid is preyed upon by various nudibranchs and we observed them concurrently, but there may be other predators too. Figure 23 illustrates one such small nudibranch feeding on Ectopleura from a posting on a nudibranch web site (Behrens 2006). It is unclear what other species feed on the hydroid but it is only present for a short period of time annually, and appears to be grazed rapidly.

Figure 22. Pink-top hydroid *Ectopleura marina* on April 25, 2004 on net panels suspended in the water by the pens. Note, this was from a submerged net panel, not a fish-growing net, managers never allow this degree of biocolonization to occur on the fish nets.

Figure 23. The nudibranch *Flabellina pricei* feeding on the pink-top hydroid, *Ectopleura marina*, from Behrens (2006).

Hiatella arctica (Nesting Clam)

The nesting or rock-dwelling clam (*Hiatella arctica*) is seen as numerous small juveniles at the study site on the netting every spring (Figure 24). The abundance of this clam on the netting at times is remarkable and impressive when viewed first hand. It never achieves its full size on the nets of course, but some of the juveniles may survive net cleaning to grow on the sea floor. It is not common in benthic samples but that is not surprising as most grab sampling devices are only efficient in soft bottoms where this clam would not be expected to survive. It has the ability to “nest” onto hard substrate or burrow into clayey sea bottoms. It may use chemical as well as mechanical means to burrow. The recurring annual sets of these clams on the nets suggests a large or nearby population in the study area.

Figure 24. Inner and outer view of shell of Hiatella arctica, the nesting clam (from J. Wooster, [http://www. jaxshells.org /wmf12.htm](http://www.jaxshells.org/wmf12.htm))

Alaria marginata (Ribbon or Winged Kelp)

Alaria marginata, sometimes known as "ribbon kelp" or "winged kelp" was the dominant contributor to biomass during summer on the anchor lines, far exceeding all other species of algae or invertebrates except bull kelp (e.g., Fig. 25). It also was seen setting on the nets in the winter and early spring and on the floats. It is very similar to another species commonly known as "wakame" in Japan where it is widely used as food. This is a potentially valuable edible species that is eaten fresh, dried or cooked in some regions of the world. The natural prevalence and abundance of this species at Cypress Island net pens suggest there may be some economic value to pursuing its culture. Unlike nutrient sensitive areas where kelp culture is being considered to remove excess nitrogen from fish farms, there is no technical, ecological reason to purposely culture seaweeds for mitigation of fish farm nitrogen discharge, as the waters are naturally rich with nitrogen, far in excess of the plant's ability to take up nutrients (Rensel Associates and PTI, 1991). This is further corroborated by our stable isotope tracing results, discussed later in this report, where uptake of feed based nitrogen was not significant.

Figure 25. Young growth of *Alaria marginata* on the net pens in late winter.

Costaria costata (Five Rib Kelp)

Costaria costata was the third most important contributor to biomass on floats, sixth on nets in spring but was only rarely found on anchor lines (Figure 26). The reason for this distribution is unclear but it may involve that nature of the holdfast. In comparison, *Alaria marginata* was highly abundant on anchor lines but it has a relatively small holdfast (Mondragon and Mondragon 2003) that may adapt to the size of anchor lines used.

Costaria costata is easily distinguishable with its five-rib blades and is an annual that varies in blade shape and texture depending on wave conditions (Druehl 2000) and possibly current velocity too, which are examples of phenotypic plasticity seen in other seaweeds and some invertebrates. Figure 26 illustrates some of the prolific growth of this common west coast seaweed on a net pen float. The species ranges from the Aleutian Islands to Monterey Bay.

Figure 26. Prolific growth of *Costaria costata* on walkway float in summer 2004.

(Historic vessel "Clam Digger" once owned by famous Seattle restaurant entrepanuer Ivar Hoagland seen in background, now serves as a feed transport and maintenance vessel)

Nereocystis luetkeana (Bull or Bullwhip Kelp)

Nereocystis luetkeana is an important kelp that occurs on all submerged surfaces at the subject fish farm but is most abundant and obvious along the shallow ends of the anchor lines (Figure 25). In some waters it grows in depths up to 30 m in depth but at Cypress Island its depth distribution is typically less, both on net pen facilities and on natural substrate. Water transparency in Bellingham Channel and Deepwater Bay where the fish farm is located is often less than would be experienced in more seaward locations such as the central or western Strait of Juan de Fuca. The Fraser River is a significant factor affecting water transparency, particularly in the spring and early summer as it creates a less saline surface layer in the region. This surface layer provides vertical stratification and a degree of stability that enhances phytoplankton production throughout the growing season of late winter through mid fall. This productivity reduces light penetration and the depth to which bull kelp grows. Strong tides also resuspend finer bottom sediments especially during spring tides, a factor that can also contribute to reduced visibility.

Figure 27. Bull kelp on anchor line at study site.

Not to be confused with giant kelp (*Macrocystis spp*.) that grows on the outer coast and parts of the Strait of Juan de Fuca, bull kelp is apparently adapted to waters of less light transparency as the blades of the plant are maintained at the top (surface) end of the plant only, not along the entire stipe or multiple stipes per plant as is the case for giant kelp.

Because of its large size and ecological importance, all live kelp was enumerated at the fish farm during mid summer and a portion of them subsampled for wet and dry weights. It occurred on all anchor lines but inexplicably was much more prevalent on up and downstream ends of the farm. Based on quick estimates, bull kelp prevalence was greater at this fish farm than the two other nearby farms. Current velocity is strongest at the study site farm, which may influence the survival and growth of bull kelp on the anchor lines. Although highly visible and abundant in the summer, its wet weight biomass was only ~1/3 that of *Alaria marginata* or only 1/5 as much in dry weight measure.

Bull kelp is reported to grow very fast (up to 15 cm/d) during the growing season and the stipe may have some commercial value as human food for pickles or salsa and the blades can be dried into chips or used in soups (Mondragon and Mondragon 2003). The quantity of kelp available on anchor lines of a typical fish farm may not be sufficient for this purpose, but among several farms there may be some potential.

Ulva and Enteromorpha (Sea Lettuce)

Ulva spp. and *Enteromorpha* spp. were recently combined into one genus (*Ulva*) despite their dissimilar appearance and morphology which in the past was differentiated into thin, flattish sheets of two cell thickness versus hollow tubes of one cell thickness, respectively, as observed at the attachment point or base. See Hayden et al. 2003 for details of this complex issue.

Figure 28. *Ulva* spp. on nets with benthic diatoms (left) and with *Alaria marginata*. (right).

Commonly referred to as "sea lettuce" the flat form grows in shallow, protected lower intertidal areas of Puget Sound and sometimes creates a nuisance when warm weather coincides with extreme low spring tides. Some species of *Ulva* may be considered possible indicators of eutrophication but as ambient DIN concentrations are high at all the Puget Sound net pen sites there is little or no chance that their growth and survival is enhanced by the fish discharge. As Ulva species show a great deal of phenotypic plasticity, it is difficult to make generalizations about what species is collected or observed in different locations. *Ulva* was not common in spring on the nets but was the 4th and 7th most important wet weight contributor on the nets during summer and winter respectively. It was insignificant on lines and floats. On nets it occurs concurrently with benthic diatom growth and with other seaweeds (Figure 28).

Gobiesox maeandricus (Northern or Flathead Clingfish)

The northern clingfish (*Gobiesox maeandricus*) was relatively commonly associated with the walkway floats, clinging to the open spaces among the colonizing organisms (Fig. 29). Its abundance is underestimated in this survey, as only those individuals remaining on the floats after removal from the water were counted. As the float removal process took some time, fish either swam away or were seen falling into the water while the crane hoisted the floats out of the water. This is a fascinating fish, able to breathe air and has a diet of crustaceans, molluscs and polychaetes, all of which are abundant on net pen floats.

Figure 29. Northern clingfish attached to the surfaces of net-pen floats after removing the float from the water and inverting.

Sabellid Polychaete "Feather Duster" Worms

The dominant invertebrate climax species of net pen anchor lines are the large, tube-dwelling "feather duster" worms that filter the water with their beautifully-colored cirri ("tentacles"). Their abundance places them as the top invertebrate on anchor and crown lines, but only those that have been in place for some extended time period. As anchor lines are replaced periodically as part of scheduled maintenance, this means that many of the sabellid worms are to be found on "crown lines" that are lines from anchors to a surface float for the purpose of moving, setting or replacing anchors (Figure 30).

Figure 30. Dense biocolonization of sabellids tube worms on a anchor "crown" line that had been in place for several years. (Svein Weise Hansen, farm co-manager alongside line).

Figure 31. Example of extended cirri of feather duster worm *Eudistylia vancouveri.*

The intertwining feather duster tubes create a varied and protected habitat for a variety of other invertebrates. Most of these sabellids appear to be the larger of the local common species, *Eudistylia vancouveri* as shown in Figure 31 with its red and maroon banded coloration. Estimates of total maximum tube length vary among sources, but we saw some that were in excess of 50 cm. A large colony of these worms would have a significant effect on clearance of particles from the water. Unlike municipal waste discharges that are often very small particles in a buoyant freshwater plume, fish farms produce mostly large, biologically labile solids particles that slowly sink and are available for benthic or epibenthic food web assimilation.

Other Species

Space and time limitations prevent a further discussion of the principal species that occur on submerged fish farm substrates. Some of the other very important species include mussels, *Mytilus edulis* as they are common on the floats and lines and would be extremely common on the nets if they were not periodically cleaned. At this fish farm, as at most other farms in Puget Sound, mussels are fed on by diving birds, especially surf scoters. Thousands of these birds reside in Deepwater Bay during the late fall to late spring every year, and from our observations and those of Washington State Dept. of Fish and Wildlife, are clearly diving to feed on mussels and other invertebrates on the pens as well in the surrounding areas. If protected by predator nets, the mussels could become a companion crop to the cultured fish, but this has not been attempted yet in Puget Sound. This could be viewed as a form of Integrated Multitrophic Aquaculture (IMTA) such as has been developed in Eastern Canada by Chopin et al. (2001) and others. This is discussed later in the section on stable isotope tracing.

6. Colonization Study Results

Introduction

The intent of the colonization portion of this study was to attempt to detect any differences of biocolonization between fish farm affected substrate and reference substrate. The working hypothesis was that fish farm affected areas should have larger, faster growing invertebrates due to the presence of solid wastes. The companion hypothesis was that seaweed, which relies on dissolved forms of nitrogen and other nutrients, would not be affected as background concentrations of macronutrients are always sufficiently replete at this (and other) fish farm location.

Results

We were able to obtain useful colonization results for the net pen floats, discussed below, but we found that biofouling of the nets was so rapid that it was impossible to sample rapidly enough with our limited funds and staff to make a meaningful estimate. The primary goal of characterizing standing stock of invertebrates and algae regardless of fish farm activities like net cleaning was met, as previously discussed, but net cleaning and fish harvesting resulted in too many variables of our selected sampling nets. So that portion of the study was documented, but not analyzed in detail. For anchor lines, we put out reference and treatment lines but the latter were lost or damaged during fish farm maintenance, so again we collected data and photographs but did not conduct a full analysis of the results as they were unbalanced between treatment and reference samples. It is important to note that the budget for this work was cut in half after commencement of the work so we had to reprioritize the work and neglect some of the initially-planned portions of the study.

Floats

After two years time submerged, the reference and control floats showed considerable growth but there was high variance in the distribution of organisms across the submerged surfaces. This was expected and had been seen in the standing stock characterization discussed above. Despite the variability, there were significant differences among treatment and reference floats for invertebrates, but not seaweed (Figure 32). The differences were not apparent in March in a set of 9 separate samples for treatment and reference but by June were significant (t test, t = 2.04, 1,6 df).

Interestingly, the increased biomass was due not only to anemones but also to a major degree due to mussels, *Mytilus edulis*. It should be noted that the total biomass of these samples was far less than those observed in the standing stock results, i.e., from floats submerged for many years (compare versus Table 5, floats). In large part, the lower biomass was due to the near complete absence of barnacles that were the most dominant invertebrate on the standing stock floats. Figure 32 indicates that seaweed may have been trending toward a significant difference too, but the small sample sizes and the lesser differences of mean biomass cast doubt on that result. Error bars represent standard error.

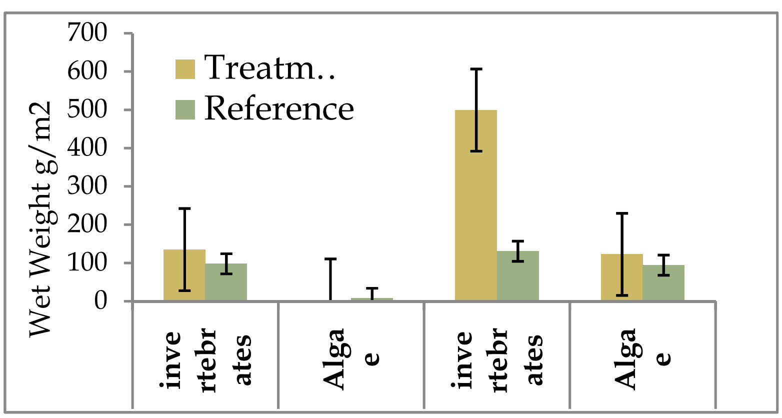


Figure 32. Wet weight colonization results for invertebrates and algae on net pen floats.

Other researchers have assessed mussel growth in the proximity of salmon pens in other countries. The results sometimes show a positive effect on mussel biomass, but not always. The data here is limited, but shows some promise. Mussels could likely be used downstream of net pens in Puget Sound with strong currents to sequester carbon-containing solids and further reduce the already very limited benthic footprint of net pens (less than 30 m in all cases, sometimes much less).

7. Stable Isotopes Assessment

Background

A dictionary definition of stable isotope is a good place to start for the uninitiated:

> *"Stable isotopes are chemical isotopes that are not radioactive. Stable isotopes of the same element have the same chemical characteristics and therefore behave almost identically. The mass differences, due to a difference in the number of neutrons, result in partial separation of the light from heavy isotopes during chemical reactions (isotope fractionation). For example, the difference in mass between the two stable isotopes of hydrogen, ^{1}H (1 proton, no neutron, also known as protium) and ^{2}H (1 proton, 1 neutron, also known as deuterium) is almost 100%. Therefore, a significant fractionation will occur. Commonly analyzed stable isotopes include oxygen, carbon, nitrogen, hydrogen and sulfur. These isotope systems have been under investigation for many years as they are relatively simple to measure. Stable isotopes have been used in botanical and plant biological investigations for many years, and more and more ecological and biological studies are finding stable isotopes (mostly carbon, nitrogen and oxygen) to be extremely useful"*.

In simple terms, in the present study we are using the ratio of "heavy" isotope of carbon or nitrogen to the normal isotope of the element in fish feed, and then observing the shift in the ratio in the fish and in any other potential consumer of the waste produced by the fish. After primary production by algae or plants, every time an organism consumes food in the food web, there is a further shift or "fractionation" of the ratio resulting in sequential and compounding shifts. These organisms do not just eat one type of food most often, so the shifts are not exact and comparable but in the future we expect to be able to make quantitative assessments for some species with defined diets.

In more technical terms, isotope ratios are reported in "delta" (δ) notation, defined as the per mil deviation from the recognized isotope standard, atmospheric N_2 for $^{15}N/^{14}N$ and Peedee Belemnite (PDB limestone) and $^{13}C/^{12}C$. These are internationally accepted standards that all laboratories use. So if there is no enrichment of heavy marine derived N (known as "MDN") for example, the δ values are low or even negative. Nitrogen isotopes are more reliable indicators or predictors of the trophic level that an organism occupies in the food web because of the large ^{15}N enrichment from one trophic level to another (Owens 1987, Peterson and Fry 1987). Thousands of studies have been done, no attempt is made here to review even the key ones.

For carbon, the results are reported as negative values, and the less negative ones represent higher degree of marine origin isotope or food web fractionation. The negative delta-C values result from the way delta notation is calculated ((Rsample/Rstandard -1)*1000, where R is the ratio of ^{13}C to ^{12}C).

It just means that almost all samples have less ^{13}C than the PeeDee belemnite standard. You could say it is the unfortunate consequence of having chosen a standard with a high ^{13}C content.

In general, the fisheries literature indicates that N is more useful than C in many study results, and it is not certain why this may be. It may be rooted in the concept that N is more scarce than C in most aquatic and terrestrial environments, so when you add some of the former, it is more likely to be used by the food web and hence a better tracer. In any event, we can use N stable isotope ratios and possibly those of carbon to see where marine derived N and C from a fish farm is being incorporated into the biota beneath and downstream of the fish farm. This gives us information as to which species and functional groups of organisms are benefiting from the waste materials of the fish farm. Additionally, the laboratory measures the amount of total N and total C in the samples, in order to estimate the ratios of stable to normal N or C.

Some bullet facts about the methodology and pertinent facts about Puget Sound include:

- Higher consumers in the marine food web concentrate proportionately higher ^{15}N relative to ^{14}N (i.e., one more electron in the stable isotope), that increases with distance from the base, primary producers (plants and algae).
- Most nitrogen produced as waste matter by salmon is in the form of ammonia, followed by a distant second by urea and trace amounts of other organic N forms (Brett and Zala 1975, Forster and Goldstein 1969). Ammonia is rapidly converted to nitrate at fish farms in Puget Sound.
- One recognized expert in the stable isotope – food web field speculated that isotopically heavy nitrogen in the fish food will be differentially retained by the fish, resetting the content of the same in excreted ammonia to a much lower level and thus affording a different signature that could be traceable (G.H. Rau, Inst. Mar. Sci., Santa Cruz, CA, pers. comm. with J. Rensel 1/2004).
- As there is very little waste N in fecal matter and both waste feed and fecal matter sinks rapidly, the spatial and isotope differences should allow for compartmentalization and traceability.

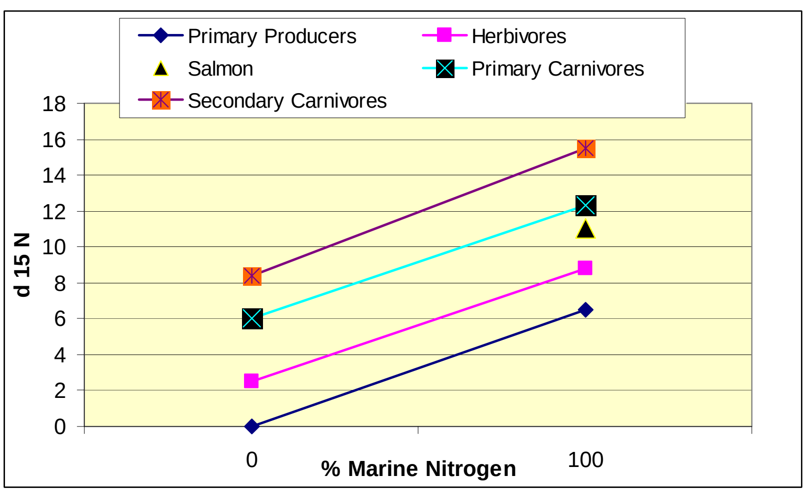


Figure 33. Example of a nitrogen trophic food web mixing model from Mathisen et al. (1988) showing ranges of possible $\delta^{15}N$ enrichment depending on level of MDN. Primary producers are often zero, indicating all N is from atmospheric sources.

It is important to understand that $\delta^{15}N$ and $\delta^{13}C$ isotopes do not pass through the food web intact, except at the beginning. As atmospheric N is fixed in primary production, there is no fractionation, hence the $\delta^{15}N$ values near 0 for algae. There is fractionation at higher steps of roughly +3 per trophic level step. The plus assigned to these values is somewhat mislead, it actually indicates a loss of heavy isotope to become less negative for nitrogen. For carbon, the effect is opposite, more negative $\delta^{13}C$ values, indicative of carbon isotope depletion, suggest an effect. This is one of the reasons that interpretation of the data can be complex. It is also an obvious reason to avoid lumping results from different trophic levels and species. There are also seasonal effects but larger, longer living organisms should be less affected by that source of variation. Figure 33 shows the possible range of effects from one particular cited study with the addition of salmon as measured in our prior studies in the Pacific Northwest (Rensel 2000, discussed below).

Stable Isotopes and Fish Farms Literature

Stable isotope monitoring (Carbon ^{13}C, Nitrogen ^{15}N and others such as Sulphur ^{34}S) offer a potentially powerful method to track the movement of waste materials from fish farms or other discharges in the food web. Surprisingly, up until a few years ago use of stable isotope tracer methods was not widely used for net-pen waste studies, with just a few limited studies available in the literature (e.g., Li-Xun et al. 1991, Tsutsumi et al. 2001). Some of these and other studies were pond studies or benthic studies not directly applicable to our bio-colonization-oriented study.

More recently an excellent paper by Yokoyama (2006) detailed sediment stable isotope composition in soft substrates in Japan that were complicated by being overlaid by an estuarine gradient. The authors were able to sort all this out, finding reduced δ13C (mean Δδ13C=−0.4‰) and enriched δ15N (mean Δδ15N=+0.9‰) values at the farm site, which reflect the deposition of C_3 (evergreen) -plant-derived and fish-derived elements, respectively. Waste feed and waste feces composition was determined in the sediments and was mapped to show exponential declines with distance from the farm sites. Areas with a mean velocity of >8 cm/s currents were shown not to have excessive deposition and build up of wastes[2] .

A study by Dolenec et al. (2006) in the Mediterranean Sea eastern Adriatic coast used stable isotope methodology to map effects of fish farms in the oligotrophic (nutrient poor) area. Water column nitrogen (DIN) varies from about 1 to 5 uM in this region versus our study farm in Puget Sound that typically has from 10 to 25 uM DIN with short term dips to 4 or 5 uM during spring blooms or river runoff effects at that time.

The authors point out that “an increase in biomass around fish farms can be expected in both oligotrophic and mesotrophic environments, irrespective of the current velocities, whereas the differences in the community structure were only recorded in oligotrophic and less dispersive environments” and they cite Cook et al. (2006) in that regard. For Puget Sound, which is naturally eutrophic in main channels and well flushed waters, this means that a possible increase in biomass around the farm sites would be expected, but possibly not if the level of natural enrichment is very high. See *Hypothesis* below for our interpretation of this. Dolenec et al. (2006) also found different ^{15}N uptake rates and compositions for different species of sponges, a result of differing internal bacteria, which results in differing geochemical maps of the distribution of effects. Clearly this is a complex matter, and with over a hundred species present at Pacific Northwest fish farms, the choice of indicator species is critical. While it is obvious that sessile, benthic invertebrates or attached seaweeds are suitable, there are so many species of both that there could be variable results among them.

[2] All Puget Sound commercial net pen sites have current velocities stronger than this, except one site which is very deep, about 50 to 60 m and much greater than the Japanese study sites of 16 to 19 m).

In the past we have used the stable isotope method to trace fish farm enhancement of fish, invertebrates and algae in middle reaches of the Columbia River (Rensel unpublished 2001). The river is the second largest river in North America but is starved for nutrient due to dam operation (i.e., it is ultra-oligotrophic) with non-detectable levels of orthophosphate year round in the study area. Water column chlorophyll is typically much less than 1 ug/L and the bottom devoid of life. But under the fish farm and immediately downstream even a casual SCUBA dive reveals a change to a diverse, populated habitat with fishes, invertebrates and periphyton effects quite obvious. Outside the effect zone it is essentially a "biological desert".

We found significant and interannually-repeatable food web effects on prickly sculpins, one of several species that are especially abundant near the farm and do not travel large distances compared to other species tested such as carp. We repeatedly observed sculpins eating wastes from the farm near the bottom, which has not been documented elsewhere in North America. Snails that inhabit the cobble and rock beneath the farm in the strong currents also showed a significant stable isotope effect.

Hypothesis

We hypothesized that the some of the biocolonization is obtaining nutrients from the fish farm wastes, especially filtering organisms. These species should demonstrate a difference in nitrogen and possibly carbon isotope composition compared to reference areas in the same locale. There are virtually no other anthropogenic sources of N and P in the locale and no estuarine gradient effect is present as was in the work of Yokoyama et al. (2006). In keeping with the standing stock hypothesis and analysis previously discussed, we will not expect seaweed at the farm site to show a nitrogen stable isotope effect, as the waters are replete with DIN and TN at all times.

The alternative hypothesis would be that there was no effect of the farm on the nutrient sequestering of the colonizing organisms of the farm. There are also intermediate results possible, where some organisms are affected and others not due to their method of feeding or nutrient uptake.

Stable Isotope Results and Discussion

Selection of species to sample was based on both abundance at the farm site and ability to find reference samples. A number of species were assayed but we had difficulty finding reference samples for some of them. Table 6 and 7 present results from species that were important in terms of biomass at the pens but also available in reference area.

These included amphipods (*Jassa marmorata*), caprellid amphipods, mussels, *Metridium senile* anemones, *Ulva* and *Alaria marginata*. Samples were taken in about equal numbers from floats and nets (except for anemones) and all surfaces were from "downstream" directions relative to the nearest concentration of farmed fish. From various prior studies we know that hatchery or commercially reared salmon and trout have a $\delta^{15}N$ content of about 12 and larger fish feed is about 10. Wild salmon usually show a $\delta^{15}N$ value of about 11 and a carbon signature of -22 to -24 (Bilby et al. 1996, Kline et al. 1990).

Table 6. Mean and sample size for nitrogen stable isotope results and res. More positive difference indicates likely effect (i.e., higher isotopic content).

Nitrogen	Treatment		Reference			
	$\delta^{15}N$	Sample N	$\delta^{15}N$	Sample N	Difference	Statistical Effect
Amphipods	10.2	9	8.3	3	1.85	yes
Caprellids	10.2	5	8.8	9	1.43	yes
Mytilus	8.9	4	7.6	6	1.24	yes
Anemone	12.7	3	13.1	4	-0.38	no
Ulva	6.5	3	7.1	4	-0.64	no
Alaria	5.4	6	6.0	3	-0.67	no

Table 7. Mean and sample size for carbon stable isotope results. More negative results indicate traceable effect (i.e., higher isotopic content).

Carbon	Treatment		Reference			
	$\delta^{13}C$	Sample N	$\delta^{13}C$	Sample N	Difference	Statistical Effect
Amphipods	-17.5	9	-18.0	3	-0.54	no
Caprellids	-18.1	5	-17.4	9	-0.72	no
Mytilus	-19.0	4	-17.1	6	-1.92	yes
Anemone	-20.6	3	-19.8	4	-0.80	No
Ulva	-19.2	3	-22.5	4	3.24	yes*
Alaria	-22.5	6	-22.9	3	0.45	No

* Significant effect, but more positive stable isotope value is indicative of no effect at the farm site.

These results in these tables and statistical testing indicate significant nitrogen effects of nutrient flow from the aquaculture operation to *Jassa* amphipods and caprellids as well as mussels. It is reasonable to assume that this is related to particulate matter from waste feces and to a much lesser extent waste feed, being available to these grazers either directly or indirectly via enhancement of benthic algae and other types of biocolonizing organisms such as hydroids.

No significant differences were noted for nitrogen isotopes of seaweed (*Ulva* or *Alaria*), which was expected as these species exclusively utilize dissolved forms of nitrogen, not solids. Surprisingly there was no effect for anemones on floats. This suggests they are utilizing other, non-fish farm sources of food and because of their long-lived nature, these other sources are likely persistent and substantial. Waste pellet analyses indicated that *Metridium senile* feeds mainly on diet of copepods, polychaete larvae, bivalve and gastropod veligers, copepod nauplii, and barnacle nauplii and cyprids (Purcell 1977 and others) and many of these plankton are the product of recruitment over a large space and time that would not be affected by a local source of nutrients like a fish farm. All of the anemones were from floats and hence are slightly further away from the farmed fish but this should not have been a major effect.

For carbon isotopes, a different result is evident. I emphasize that this is confusing; one has to remember that a more negative measurement than the reference sample indicates an effect, i.e., carbon isotope depletion. There were no significant differences between reference and treatment except for mussels and *Ulva* seaweed (Table 7) but it is important to note that mussels showed a positive effect while Ulva in reference stations had the reverse effect. The mussels were apparently benefiting from the particulate carbon associated with solid wastes. The Ulva results are inexplicable at this point. If Ulva at the farm site was benefiting from carbonate from the fish farm operation we would have expected more negative C isotope content, but it was the reverse. But the fact remains that no effect was observed for nitrogen, the nutrient likely to be the most limiting in comparison to all others.

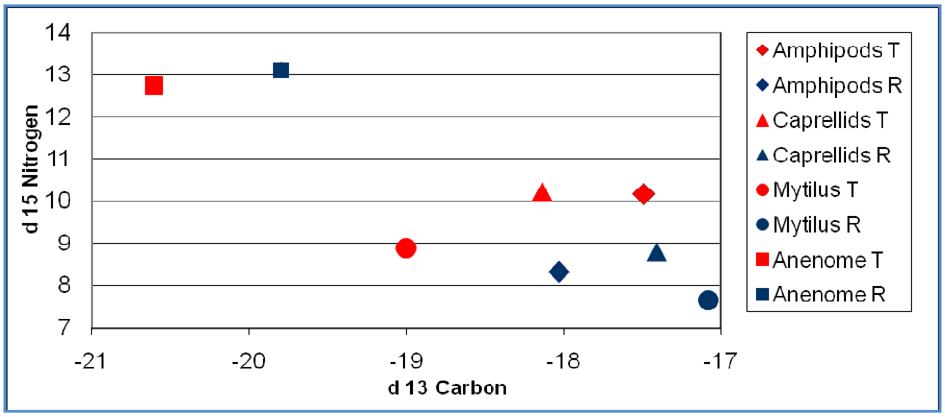


Figure 34. Dual isotope plot of N and C stable isotopes for invertebrate species. The further the distance the greater the effect vertically (for N) and horizontally (for C) between treatment (red) and reference (blue) samples.

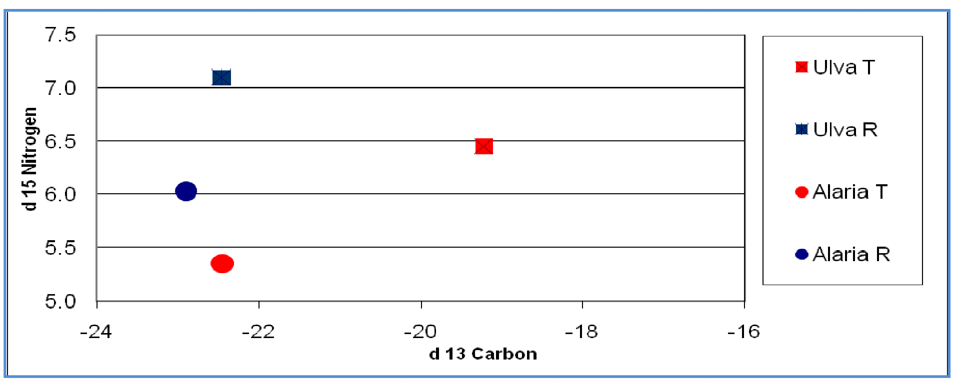


Figure 35. Dual isotope plot of N and C stable isotopes for invertebrate species. The further the distance the greater the effect vertically (for N) and horizontally (for C) between treatment (red "T") and reference (blue "R") samples.

Ulva was never highly abundant on the fish farm substrates, but as discussed above was relatively more abundant on the nets during summer and again in winter (actually late winter with the increasing photoperiod and onset of the new annual growing season). A very large percentage of the carbon flow from fish feed ends up as respired carbon dioxide (~50%) and this large source could be helping fuel the growth of seaweed or macrophytes in some cases. Yet there was no significant C depletion effect on either *Ulva* or *Alaria marginata* (a more abundant and brown seaweed). Equal samples of both were from nets or floats, so distance and dilution are not likely an effect. There are large differences in the physiological ecology of *Ulva* (a fast growing colonizer green algae) versus *Alaria* that could account for these differences.

Figures 34 and 35 are dual isotopic plots that express the same information as Tables 6 and 7 in a graphic form to illustrate differences and similarities between treatment and reference samples by species.

These results tend to validate our initial hypothesis that waste nitrogen would not influence seaweeds in the area. Seaweeds tend to have higher ½ saturation constants than phytoplankton (i.e., the level at which growth is limited to 50% of maximum) but the concentrations at the study site are almost always well in excess of such levels.

There was a measurable effect of waste nitrogen on invertebrates including amphipods, caprellids and mussels. The first two could possibly be explained through direct or indirect effects, i.e., from consumption of small waste particles directly or through use of macroscopic biocolonizers such as benthic algae and diatoms or protozoans. As mussels are filter feeders, they must be filtering the water for particulate nitrogen, despite the fact that most waste nitrogen is produced in the dissolved form.

Stable Isotope Summary

From the above it is clear that there were some useful results, and that the effects of the pens may be determined through nitrogen and possibly carbon stable isotope analysis. The analysis is not complete. It is possible to use an isotope mixing model to estimate contributions of waste feed or waste feces to individual species if we had accurate, direct measurements of waste fecal matter and feed. The latter is no problem, the former could be as it requires data collection from fish held in controlled tank conditions (e.g., Yokoyama et al. 2006).

It may also be able to prepare geochemical maps of the extent of the effects on sessile organisms such as benthic infauna. The cost of stable isotope analysis continues to decline and the number of laboratories conducting the work is increasing. It is likely that stable isotope methodology will become the most common and prevalent means of researching the effects of fish farms in some future time. It is the most promising means to determine food web effects and it may be possible to determine effects on higher food web species such as fish and marine birds. There remain unanswered questions, but the more we learn about local food webs the better we can adapt the stable isotope tool to individual regions.

8. Sea Bird Use

For several decades fish farmers and others have noticed that marine birds tend to accumulate near most of the commercial net pens in Puget Sound. This tendency of course has been noticed by agency managers of non game wildlife too (D. Nysewander, Wash. Dept of Fish and Wildlife, pers. comm. March 2007). It is most likely this accumulation is due to the halo of enhanced food production on and around the cages that was previously discussed. The most prevalent birds are surf scoters, a species that migrates up and down the North American west coast and has relatively high winter site fidelity (Figs 36 and 37).

Unfortunately, the population of these birds has been declining over the past 25 years along the entire west coast, for unknown reasons (Nysewander et al 2005). Sea ducks in general are considered the least studied group of North American waterfowl. The Washington State Department of Fish and Wildlife and cooperating universities have been conducting surveys of “locations of the spring staging, summer nesting, and fall molting grounds need to be documented, as well as the migratory paths from, and back to, the wintering grounds” as a means to better understand these birds and detect means to maintain their survival. We began counting these birds in Deepwater Bay and near the fish farms in the winter of 2004-05 and found total counts of diving ducks that averaged ~1,800 birds/day within a 3 km radius. In early morning most of the birds may be found very near one or all three of the fish farms in the bay. Nearby bays such as Eagle Harbor and the associated bight had very few or no birds on most occasions. Most of the observed birds were surf scoters, along with some western grebes, a few eared grebes and even a flock of harlequin ducks that lives at the subject farm site. By the winter of 2006 the numbers in the bay had declined to less than 500 birds (Figure 38). This could be due to interannual variation, but no one knows.

Scoters are voracious consumers of mussels and clams and must be separated from mussel rafts, but to fish farmers they help keep the nets and floats clean. They clearly benefit from the biota around the pens, although the exact prey items have not been studied. If you have any doubt about the above, you simply need to visit one of the fish farm sites in winter and observe the birds feeding. A flock of 50 or more birds will often line up parallel to a pen, swim toward the pen, dive and disappear for a few minutes. Suddenly they pop up swimming away from the pens after a few minutes, only to repeat the operation again and again.

Figure 36. Some of the hundreds of birds present immediately adjacent to the fish farms throughout the fall through spring period. These birds are feeding on bottom organism that are enhanced by the presence of the farm in this well flushed area.

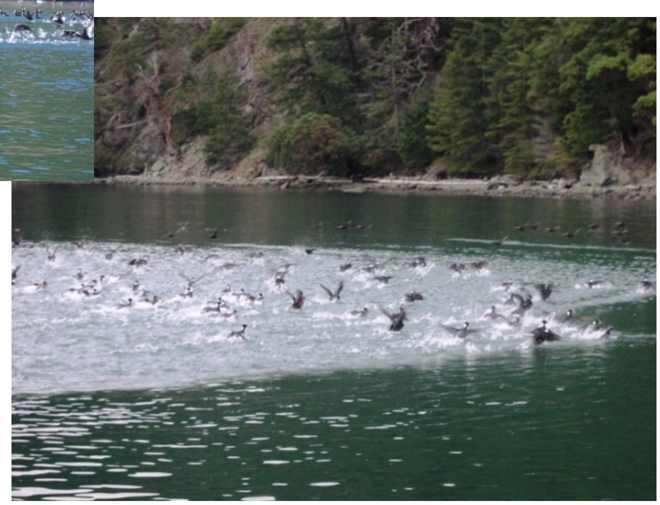


Figure 37. Surf scoters alongside the pens during late November 2004.

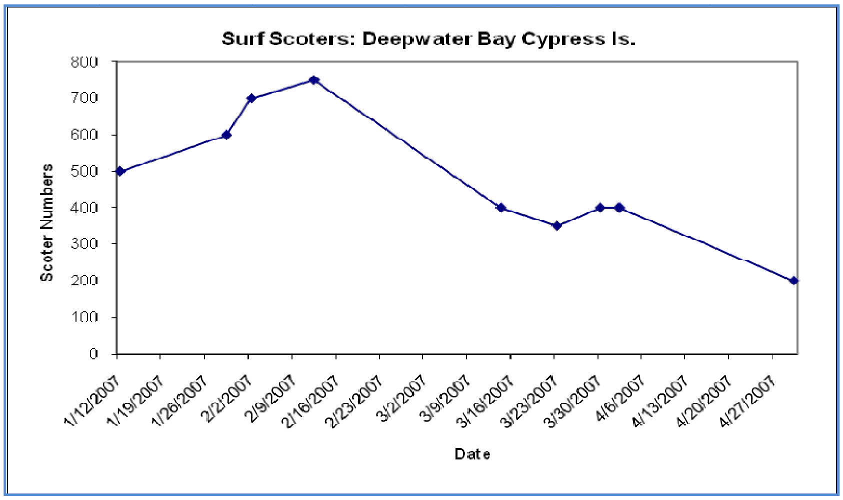


Figure 38. Surf scoter count in Deepwater Bay near fish farms in 2007, courtesy of Brandon Jensen, AGS.

9. Discussion

This report demonstrates that invertebrates and seaweed associated with net pens constitute a locally substantial concentration of biological diversity and biomass approximating a "floating reef". The biota also provides a degree of trapping and filtering of fish farm waste products not previously investigated or quantified for the Pacific Northwest. Stable isotope food web studies have occurred in other countries with differing nutrient and food web conditions and it is apparent that there is considerable variability among regions. Most of the studies related to net pen aquaculture have been in the Mediterranean Sea, and area of very low natural nutrient levels and susceptible to adverse changes from any kind of large, nearshore nutrient discharge. However, nutrients are essential for marine life and it is a common but serious mistake to assume that all sources of nutrients are somehow unwarranted and a form of pollution.

In the eastern Mediterranean Sea for example, the Nile River has been an nutrient important source for stimulating and maintaining marine fisheries for millennia. Alterations of river flow from the Aswan High Dam, heavy metal pollution from on shore industries, and anthropogenic nutrient loading have brought about numerous adverse changes in the river and coastal environment of that area (Nixon 2003, Mikhail et al. 2006, Ibrahim and El-Naggar 2006).

Along the west coasts of South and North America, upwelling of nutrients from the deep ocean are in part responsible for maintenance of plankton and fish stocks throughout the region. It is a popular misconception that nutrients are a problem, which can be traced to the problems that have been created in estuarine environments where adverse effects such as algal blooms, resulting oxygen depletion when blooms crash and other problems are indeed a concern.

This study provides a first step in that direction for Pacific Northwest waters and should be of interest to net-pen planners, operators, regulators and engineers. The data confirm our hypothesis that several types of invertebrates are benefiting from the wastes and habitat creation. Seaweeds benefit from habitat creation, but there is no indication that they are gaining a significant amount of their nutrient supply from the fish farm, which was expected. Again, all commercial fish farms in Puget Sound are in non-nutrient sensitive waters where light, not nutrient supply, limits primary productivity. Several types of invertebrates are directly benefiting from the waste products of the farm and the stable isotope methodologies holds promise to further measure and understand the true extent of the positive food web halo that exists at well-located and operated fish farms.

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